



Chapter 12

CHEMICAL REGULATORS OF GIBBERELLIN STATUS AND THEIR APPLICATION IN PLANT PRODUCTION

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Abstract: Gibberellins and inhibitors of their biosynthesis are widely used in modern agriculture, horticulture and viticulture. Their global market is in the range of US\$ 500 million. The gibberellins GA₃, GA₄ and GA₇ are primarily used to increase fruit yield and/or quality in fruit trees and table and wine grapes. Distinct steps of the gibberellin biosynthetic pathway can be inhibited by growth retardants: chlormequat and mepiquat chloride, ancymidol, flurprimidol, paclobutrazol, uniconazole, and the fungicides tebuconazole and metconazole and daminozide, trinexapac-ethyl and prohexadione-calcium are currently used in crop production. Growth retardants reduce shoot elongation, thereby lowering the risk of lodging in cereals, rice and oilseed rape, and making ornamentals more compact. A better canopy structure with improved formation of reproductive structures is induced in cotton and peanuts. In fruit and nut trees, less pruning is required and increases in crop yield and quality are obtained.

Keywords: Gibberellins, biosynthesis inhibitors, growth retardants, plant growth regulators, agriculture, horticulture, viticulture, crop production, canopy structure, lodging

12.1 Introduction

Plant growth regulators (PGRs) (often also referred to as plant bioregulators) can be defined as natural or synthetic compounds that affect developmental

or metabolic processes in higher plants in a desired way. They are not phytotoxic, play no role as a plant nutrient and are, typically, applied at low dosages. In most cases, PGRs affect the plant's hormonal status. PGRs can significantly improve the performance of crop plants and their effects are often targets for breeding, be it by conventional methods or by employing genetic engineering. However, in spite of the possibilities that breeding offers, PGRs often enable faster and better solutions to many problems. Most importantly, they allow for an active regulation of plant processes, thereby adjusting a given genotype to its particular growing conditions. In other words: plant growth regulators can be used highly flexibly for the fine-tuning of crop plants that grow at a given site under largely uncontrollable and unpredictable environmental conditions. Overviews and detailed information on uses of plant growth regulators in different crops are given in Weaver (1972), Martin (1983), Nickell (1983), Schott and Walter (1991), Hedden and Hoard (1994), Gianfagna (1995), Thomson (1999), Fletcher *et al.* (2000), Basra (2000), Petracek *et al.* (2003), Rademacher (2004a) and Rademacher and Brahm (2010).

In the global crop protection market of approximately US\$ 56.7 billion (€ 42.7 billion) of annual sales worldwide in 2014 (Anonymous, 2015a), PGRs play a relatively small role and account for only some 2.5% of the sales (Rademacher, 2010a). According to rough estimations, inhibitors of gibberellin (GA) biosynthesis and GAs *per se* account for 40% and 15% of the PGR market, respectively. Very few companies are still engaged in active research on GAs or inhibitors of GA biosynthesis. Due to mounting public concerns about the safety of agrochemicals, guidelines for developing and using such products have become increasingly restrictive. PGRs do not only have a relatively small market, they are also significantly more complex to work with and above-average investments are required for research, development and market introduction. In most cases, herbicides, fungicides or insecticides are less complicated and, thus, appear better suited for investments (Rademacher, 2010a). In order to be economically viable, a new PGR must have a sufficiently large market and be sold at a price offering sufficiently attractive profitability. As a consequence, the industry has to concentrate its efforts on uses in 'big crops', which are primarily represented by wheat, rice, maize, sugar cane, soybeans, oilseed rape, cotton, apples, citrus fruits, grapevines and bananas. Developing a new plant growth regulator for a 'minor crop' is, typically, not attractive for a company and will not be pursued. To overcome this obstacle, minor uses can be made possible in most countries by employing certain programs, such as IR-4 in the USA. However, a prerequisite for approaches of this type is a valid registration of the candidate product in at least one 'major crop'.

Two main approaches to changing the levels of biologically active GAs are of current practical relevance: (1) increasing endogenous GA levels by applying additional amounts of GAs and (2) reducing endogenous GA concentrations by treatment with inhibitors of GA biosynthesis. In the following parts, such types of PGRs will be dealt with. Emphasis will

be placed on compounds and the derived products that are of current use in plant cultivation. Common names will primarily be used for addressing distinct compounds. Such names are assigned by the International Organization for Standardization (ISO) and are short, distinctive, non-proprietary and widely accepted. Systematic chemical names will also be given for most compounds mentioned following the nomenclature rules of the International Union of Pure and Applied Chemistry (IUPAC). Of the product names, only the original and most widely used will be mentioned. Likewise, only key producers or main providers will be named. Much more information on such and related subjects is given in pertinent reference books, e.g. Anonymous (2012) and Anonymous (2014). Useful information is also provided on different sites in the internet, e.g. the Crop Protection Database of Farm Chemicals International: <http://www.farmchemicalsinternational.com/crop-protection-database/#/>; the database of pesticide physicochemical and ecotoxicological data provided by the University of Hertfordshire in the UK: <http://sitem.herts.ac.uk/aeru/iupac/>; the EU pesticide database, which, *inter alia*, indicates the registration status and maximum residue levels in plant products for EU member states: http://ec.europa.eu/sanco_pesticides/public/?event=homepage; the crop production database of the Food and Agriculture Organization of the United Nations, Statistics Division (FAOSTAT) <http://faostat3.fao.org/compare/E>.

12.2 Gibberellins

The main hormonal functions of GAs in higher plants are the promotion of longitudinal growth, the induction of hydrolytic enzymes in germinating seeds, the induction of bolting in long-day plants, and the promotion of fruit setting and development. Accordingly, a number of uses of GAs in crop production have become general practice. In addition, there is also considerable use to accelerate the process of malting for beer-making.

In the early 1950s, when the work of Japanese scientists on GAs from the fungus *Gibberella fujikuroi* became known in other countries, companies in the UK and in the USA started to work on both GA production by fermentation and their application on crop plants. Work at ICI's Akers Laboratories in the UK on fermentation and chemical identification yielded the first production patents in 1954. Parallel, agricultural uses for GAs were investigated at ICI's Jealott's Hill research facility. By late 1957, ICI had granted manufacturing and use licenses to Abbott Laboratories, Merck & Co., Inc., Eli Lilly & Co. and Pfizer, Inc. in the USA and to Takeda Chemical Industries in Japan (Martin, 1983; Petracek *et al.*, 2003). Syngenta (into which ICI has merged) is still a major distributor of GA preparations. Valent BioSciences Corporation emerged from Abbott Laboratories and is now part of Sumitomo Chemical

Co., Ltd. In addition to selling GA-containing products, Valent BioSciences is still actively engaged in finding new uses and improved formulations of GAs. Currently, Valent BioSciences lists several dozens of uses in plant production for its different GA preparations.

Fermentations of *G. fujikuroi* are used to produce GA₃ and a mixture of GA₄ and GA₇ on a commercial scale. A chemical synthesis of GAs is possible (Mander, 2003). However, it is highly complex and much too expensive for any commercial exploitation. Likewise, testing other GA-producing fungi did not result in competitive alternatives. Production of pure GA₄ might have been achieved, for instance, with the fungus *Sphaceloma manihoticola*, which causes the superelongation disease of cassava (Rademacher and Graebe, 1979). However, attempts made at ICI, Norsk Hydro and BASF failed because of difficulties to establish competitive large-scale fermentations (Rademacher, unpublished). The phthalimide-type compound AC 94,377 [1-(4-chloro-1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)cyclohexane-1-carboxamide], a relatively simple chemical structure with approximately 5 to 10% of the activity of GA₃ (Los *et al.*, 1980; Yalpani *et al.*, 1989; Rademacher, unpublished), was discovered and developed in the agricultural branch of former American Cyanamid Company (now part of BASF). It was sold under the trade name Surestem for a short time for the elongation of stems of roses (Kust, 1993), but for commercial reasons, it is no longer available. Currently,

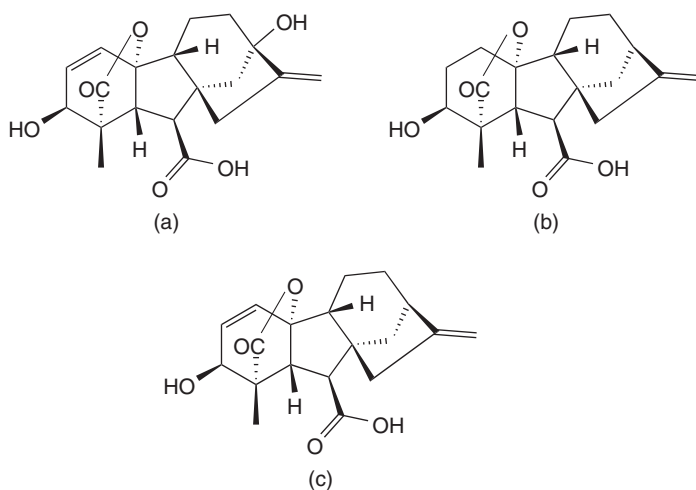


Figure 12.1 Chemical structures of (a) gibberellin A₃ = gibberellic acid = GA₃ = (3*S*,3*aR*,4*S*,4*aS*,7*S*,9*aR*,9*bR*,12*S*)-7,12-dihydroxy-3-methyl-6-methylene-2-oxoperhydro-4*a*,7-methano-9*b*,3-propeno[1,2-*b*]furan-4-carboxylic acid, (b) gibberellin A₄ = GA₄ = (3*S*,3*aR*,4*S*,4*aR*,7*R*,9*aR*,9*bR*,12*S*)-12-hydroxy-3-methyl-6-methylene-2-oxoperhydro-4*a*,7-methano-3,9*b*-propanoazuleno[1,2-*b*]furan-4-carboxylic acid and (c) gibberellin A₇ = GA₇ = (3*S*,3*aR*,4*S*,4*aR*,7*R*,9*aR*,9*bR*,12*S*)-12-hydroxy-3-methyl-6-methylene-2-oxoperhydro-4*a*,7-methano-9*b*,3-propenoazuleno[1,2-*b*]furan-4-carboxylic acid.

the majority of GAs sold globally originates from China. Several Chinese companies indicate on their websites huge supply capacities for GA₃. Prices around US\$ 200.00 per kilogram for bulk quantities (>90% purity) of GA₃ are typical. Total global annual use of GA₃ is in the range of 100 tons with approximately three quarters of this used in plant production, the rest in the beer-brewing industry.

GA₃ is among the most widely used PGRs. It has found many applications in viticulture, horticulture and agriculture, e.g. in the production of seedless table grapes, in berry thinning of wine grapes, in improving citrus fruit quality, in increasing fruit size in pears and sweet cherries, and in accelerating seed germination. GA₄ is less persistent than GA₃ and GA₇ and is, therefore, better suited where too long-lasting effects are unwanted. However, due to the close chemical similarity of GA₄ with GA₇, their separation in fermentation extracts from *G. fujikuroi* is very difficult. As a result, the content of (mostly unwanted) GA₇ in different commercially available preparations varies between approximately 40% and insignificant amounts. The main use of GA_{4/7} is to reduce fruit russetting in apple. The structures of the commercially available GAs are shown in Figure 12.1.

12.3 Inhibitors of gibberellin biosynthesis

The biosynthesis of GAs in higher plants is relatively well understood. A rough outline of the steps involved and the points of inhibition by the different growth retardants dealt with in this chapter is shown in Figure 12.2. GA formation can be separated into three stages, according to the nature of the enzymes involved and the corresponding localisation in the cell: (1) terpene cyclases catalyse the formation of *ent*-kaurene via *trans*-geranylgeranyl diphosphate and *ent*-copalyl diphosphate (CDP) in proplastids, (2) cytochrome P₄₅₀-dependent mono-oxygenases associated with membranes of the endoplasmic reticulum are involved in the steps leading from *ent*-kaurene to GA₁₂, (3) dioxygenases, which are located in the cytosol and which require 2-oxoglutarate as a cosubstrate are required for the subsequent hydroxylations into different GAs. More details on GA biosynthesis can be found in Hedden and Kamiya (1997), Yamaguchi (2008), Sponsel and Hedden (2010), Hedden (2012), Hedden and Thomas (2012), and in Chapter 2 of this volume.

Inhibitors of GA biosynthesis lead to less cell elongation and cell division, thereby making plants more compact, which may have a range of benefits in crop production. Such compounds are often referred to as 'growth retardants'. Several inhibitors of GA biosynthesis are known, some of which are used in crop production (Rademacher, 2000; Rademacher and Brahm, 2010). These compounds represent the most important group of PGRs, both in terms of commercial value and of treated area. The site of interaction of

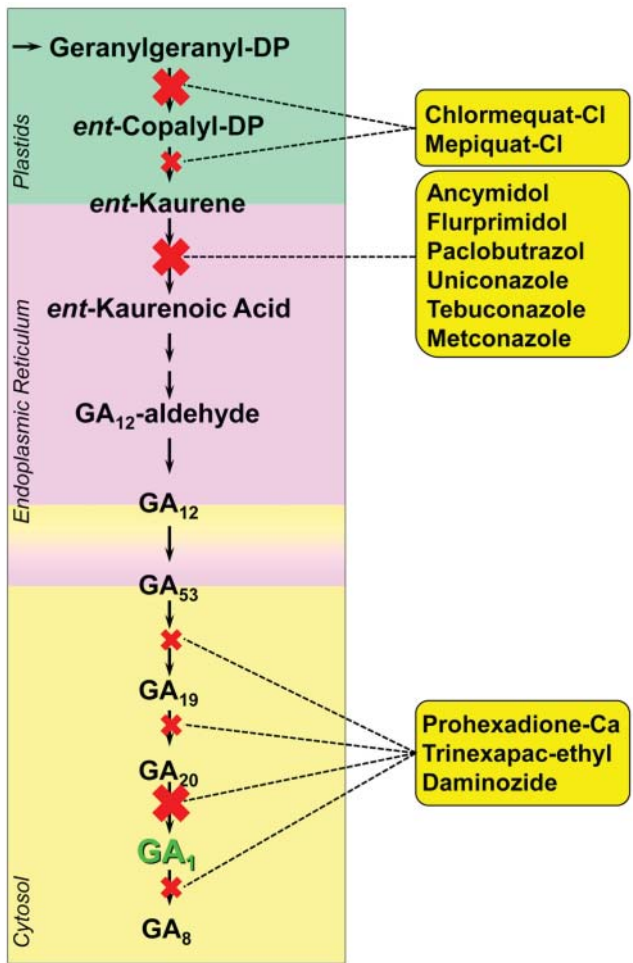


Figure 12.2 Main steps of gibberellin biosynthesis leading to biologically active GA₁ and points of inhibition by plant growth retardants. The cellular locations of the reactions is indicated by different greyscales. (The conversion of GA₁₂ into GA₅₃ can be located in both the endoplasmic reticulum or the cytosol.)

growth retardants with distinct steps in the biosynthetic sequence of GA formation has primarily been elucidated by using cell-free enzyme systems prepared, for instance, from *G. fujikuroi* or from immature pumpkin or pea seeds. Analysing the spectrum of GAs and their precursors from fungal cultures or intact plants treated with inhibitors has also been helpful for this purpose. Overviews on the biochemical mode of action of growth retardants have been given by Hedden (1990) and Rademacher (2000). Since that time, little additional information has become available on this subject.

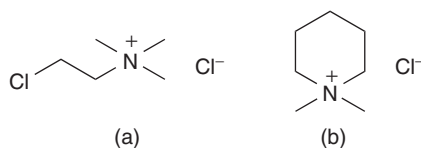


Figure 12.3 Chemical structures of (a) chlormequat chloride = chlorocholine chloride (CCC) = 2-chloroethyltrimethylammonium chloride and (b) mepiquat chloride = 1,1-dimethylpiperidinium chloride.

12.3.1 Quaternary ammonium compounds

Several compounds that possess a positively charged ammonium, phosphonium or sulfonium group inhibit cyclases involved in early stages of GA biosynthesis, thereby blocking the formation of *ent*-kaurene. Out of these, the quaternary ammonium compounds chlormequat chloride and mepiquat chloride (Figure 12.3) are of practical relevance. For more 'onium-type' representatives see Rademacher (2000).

Chlormequat chloride and related compounds inhibit CDP-synthase, both in the GA-producing fungus *G. fujikuroi* and in cell-free preparations of this fungus and of higher plants. *ent*-Kaurene synthase is also inhibited, but mostly at a lower degree of activity (Shechter and West, 1969). To obtain any significant effects in cell-free preparations, relatively high concentrations of chlormequat chloride have to be used and, in some cases, the compound is even inactive (Anderson and Moore, 1967; West, 1973; Frost and West, 1977; Hedden, 1990). The same is true of mepiquat chloride: in an enzyme system derived from pumpkin (*Cucurbita maxima*) endosperm, concentrations as high as 10^{-3} M of this compound, as well as of chlormequat chloride, did not affect the spectrum of GAs and GA precursors (Hildebrandt, 1982). A possible explanation for this lack of activity could be the fact that these compounds are almost inactive in intact pumpkin plants and this may also be expected for corresponding cell-free preparations. Consequently, chlormequat chloride has been tested with enzymes derived from germinating wheat seedlings, where it gave pronounced effects (Graebe *et al.*, 1992). Chlormequat chloride lowered the levels of GA₁ in both the shoots and grains of *Triticum aestivum* (Lenton *et al.*, 1987). Likewise, it led to a dose-dependent reduction of all GAs (GA₁₂, GA₅₃, GA₄₄, GA₁₉, GA₂₀, GA₁, GA₈) present in two cultivars of *Sorghum bicolor* (Lee *et al.*, 1998). In *Eucalyptus nitens*, it caused a reduction of GA₂₀ and GA₁ (Williams *et al.*, 1999).

Chlormequat chloride

Chlormequat chloride was first described in 1960 by N.E. Tolbert from Michigan State University at East Lansing in the USA to reduce shoot length in several plant species (Tolbert, 1960a, 1960b; Wittwer and Tolbert, 1960). The commercial rights were held at that time by American Cyanamid Company. The significance of using chlormequat chloride as an anti-lodging agent in

intense European wheat production was soon recognised (Linser *et al.*, 1961; Jung and Sturm, 1964). It was introduced under license as Cycocel[®] in Germany by BASF in 1965. After 50 years, this growth retardant is still the most widely used PGR in cereal production, particularly in wheat, rye, triticale and oats. Together with uses in other cultivated plants, it is, in terms of treated area, the number one PGR on a global scale.

Mepiquat chloride

After having success with chlormequat chloride, BASF developed another quaternary ammonium compound: mepiquat chloride (Zeeh *et al.*, 1974). After its introduction in 1979, mepiquat chloride became a very successful PGR, particularly in cotton. Alone or in combination with other PGRs, it is also used in other crops.

Mepiquat has been detected in samples of processed plant material, such as roasted coffee beans, roasted barley seeds, crust of bread and alfalfa pellets, even if any previous use of mepiquat chloride or other sources of contamination could be ruled out. Recent work involving coffee beans and barley seeds indicates that such findings result from a Maillard-driven degradation of lysine under dry thermal conditions and in the presence of naturally occurring trigonellin (Wermann *et al.*, 2014). Pipecolatebetain, which is structurally close to mepiquat and which occurs at relatively high concentrations in most vascular plants (Rhodes and Hanson, 1993) may also serve as a source of mepiquat under such processing conditions.

12.3.2 Compounds with a nitrogen-containing heterocycle

Distinct pyrimidines, 4-pyridines, norbornanodiazetins, imidazoles and triazoles inhibit GA formation (Rademacher, 2000). Out of these, ancymidol, flurprimidol, paclobutrazol, uniconazole and its (*E*,3*S*) isomer uniconazole-P (Figure 12.4) are of practical relevance. The triazole-type fungicides tebuconazole and metconazole induce a clear growth-retarding effect particularly in oilseed rape and are used both as fungicides and PGRs in this crop. These growth retardants act as inhibitors of cytochrome P450-dependent mono-oxygenases, which catalyse the oxidative steps from *ent*-kaurene to *ent*-kaurenoic acid and which are primarily located in the endoplasmic reticulum (Graebe, 1987 and references cited therein; Miki *et al.*, 1990). Steps lying after *ent*-kaurenoic acid, which may still involve mono-oxygenases, do not seem to be affected (Graebe, 1987). The structural feature common to all these inhibitors of *ent*-kaurene oxidation is a lone electron pair on the sp²-hybridised nitrogen of their heterocyclic ring. In each case, this electron pair is located at the periphery of the molecule (Rademacher *et al.*, 1987) and it appears likely that it displaces oxygen from its binding site at the protoheme iron (Coolbaugh *et al.*, 1978). Evidence for such a type of interaction has been presented for ancymidol in microsome preparations of *Marah macrocarpus* (Coolbaugh and Hamilton, 1976; Coolbaugh *et al.*, 1978) and for BAS 111..W

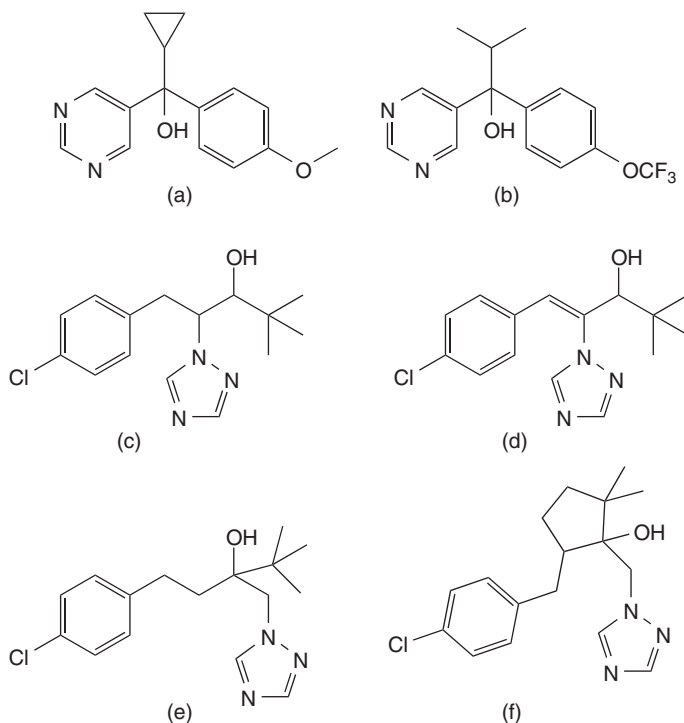


Figure 12.4 Chemical structures of (a) ancymidol = (*RS*)- α -cyclopropyl-4-methoxy- α -(pyrimidin-5-yl)benzyl alcohol, (b) flurprimidol = (*RS*)-2-methyl-1-pyrimidin-5-yl-1-(4-trifluoromethoxyphenyl)propan-1-ol, (c) paclobutrazol = (*2RS,3RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl)pentan-3-ol, (d) uniconazole = (*E*)-(*S*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl)pent-1-en-3-ol, (e) tebuconazole = (*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol and (f) metconazole = (1*RS,5RS*;1*RS,5SR*)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol.

(an experimental triazole-type PGR), using microsomal membranes isolated from immature pumpkin endosperm (Luster and Miller, 1993).

Depending on the presence or absence of a double bond, uniconazole-P and paclobutrazol possess one or two asymmetric carbon atoms, respectively. Since commercial paclobutrazol consists mainly of the *2RS,3RS* diastereoisomer (Sugavanam, 1984), this structure allows virtually only two enantiomers, as does uniconazole-P. Detailed experiments carried out with the optical enantiomers of paclobutrazol have shown that the *2S,3S* form exhibits more pronounced plant growth-regulatory activity and blocks GA biosynthesis more specifically, while the *2R,3R* enantiomer is more active in inhibiting sterol biosynthesis (Sugavanam, 1984; Hedden and Graebe, 1985; Burden *et al.*, 1987). Fungicidal side activities of paclobutrazol are attributed to its effect on fungal ergosterol formation (Sugavanam, 1984).

It has been demonstrated that the 2*S*,3*S* enantiomer is structurally similar to *ent*-kaurene, whereas the 2*R*,3*R* form is closely related to lanosterol, the respective intermediates of GA and ergosterol biosynthesis (Sugavanam, 1984). Similar chiral specificities have been found for uniconazole-P (Izumi *et al.*, 1985) and further related compounds, such as triapenthenol (Lürssen, 1987) and inabenfide (Miki *et al.*, 1990): in all cases, the *S* enantiomer was more inhibitory to *ent*-kaurene oxidation than the respective *R* counterpart. Using computer-assisted molecular modelling methods, clear structural similarities between the norbornanodiazetin tetracyclacis and the growth-retarding forms of paclobutrazol and uniconazole-P with *ent*-kaurene and *ent*-kaurenol could be demonstrated (Sugavanam, 1984; Müller *et al.*, 1987; Katagi *et al.*, 1987). This indicates that, within limits, distinct structural features are required to bind to and thereby block the active site of the enzyme. One may assume that the structures of the other growth retardants possessing an N-containing heterocycle also fit into this scheme.

Clear evidence is available that reduction of shoot growth caused by pyrimidines, 4-pyridines, norbornanodiazetins, imidazoles and triazoles is due to a lowered content of biologically active GAs. Reduced levels of such GAs have, for instance, been analysed by modern techniques under the influence of ancymidol in beans (Shive and Sisler, 1976), paclobutrazol in barley and wheat (Lenton *et al.*, 1987) and in *Eucalyptus nitens* (Williams *et al.*, 1999) and uniconazole-P in rice (Izumi *et al.*, 1984) and *Sorghum bicolor* (Lee *et al.*, 1998).

Ancymidol and flurprimidol

Ancymidol (Tschabold *et al.*, 1970) and flurprimidol (Barrett, 1982) are closely related in structure. These pyrimidines were introduced by Elanco Products (now part of Dow AgroSciences) in 1971 and 1989, respectively. SePRO Corporation has, meanwhile, become a major distributor. The compounds are used to decrease the rate of growth in a wide range of mono- and dicotyledonous species, including perennial turf grasses, ornamental cover species, herbaceous and woody ornamentals, and deciduous and coniferous trees grown in gardens and parks. There are no registrations in plants used for human or animal nutrition.

Paclobutrazol, uniconazole and uniconazole-P

Paclobutrazol was first reported as a new and very potent PGR by Lever *et al.* (1982). Market introduction by ICI Agrochemicals (now part of Syngenta) was in 1986. The first international publication on uniconazole was by Izumi *et al.* (1984). The closely related paclobutrazol, uniconazole and uniconazole-P are very persistent, with an average half-life of approximately six months, both in plants and in the soil. They are used particularly in countries with warmer climates to control vegetative growth of fruit trees such as avocados, mangos or litchis. Lodging control in rice and the production of more compact ornamentals are further uses of these compounds.

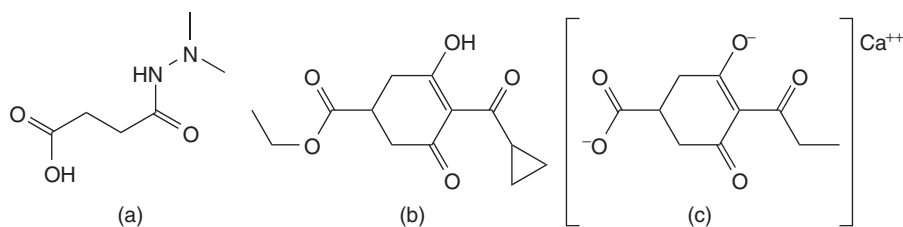


Figure 12.5 Chemical structures of (a) daminozide = *N*-(dimethylamino)succinamic acid, (b) trinexapac-ethyl = ethyl (*RS*)-4-cyclopropyl(hydroxy)methylene-3,5-dioxocyclohexanecarboxylate and (c) prohexadione-calcium = calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate.

Tebuconazole and metconazole

In addition to blocking ergosterol biosynthesis in fungi, some triazole-type fungicides may also block GA biosynthesis in distinct plant species. This is of practical interest for using tebuconazole and metconazole to control shoot growth in oilseed rape. In Germany, metconazole is additionally in use as a PGR for ornamentals. Kuck and Berg (1986) from Bayer AG reported first on tebuconazole, which was commercialised as a fungicide starting in 1988. Metconazole was discovered by Kureha Chemical Industry Co., Ltd. in 1986 with the first international report by Sampson *et al.* (1992). The compound was jointly developed with Shell International and, later, American Cyanamid Co. and BASF AG. Market introduction (first as a fungicide, then, additionally, as a PGR) was by Cyanamid Agro in France in 1994.

12.3.3 Structural mimics of 2-oxoglutaric acid

This group is represented by the acylcyclohexanediones prohexadione-calcium and trinexapac-ethyl. Also daminozide, a succinic acid derivative, falls into this category (Figure 12.5).

The free acids prohexadione and trinexapac represent the active forms of the respective calcium salt and ethyl ester. Prohexadione is formed immediately from its calcium salt upon dissolving in water. In contrast, trinexapac-ethyl has to be saponified via biochemical processes, which may lead to delays in the onset of action, particularly when weather conditions are unfavourable (Rademacher, 2014). Prohexadione and trinexapac block soluble 2-oxoglutarate-dependent dioxygenases involved in late steps of GA biosynthesis. Studies with cell-free preparations have revealed that most steps after GA_{12} are inhibited by prohexadione and other acylcyclohexanediones (Nakayama *et al.*, 1990b, 1991; Graebe *et al.*, 1991; Griggs *et al.*, 1991; Hedden, 1991; Kamiya *et al.*, 1992; Rademacher *et al.*, 1992). Enzyme kinetic data indicate that the retardants act competitively with respect to 2-oxoglutarate, a cosubstrate for these enzymes (Griggs *et al.*, 1991; Hedden, 1991). GA 3-oxidase, which catalyses hydroxylations at position 3β (e.g.

the formation of GA₁ from GA₂₀) and also GA 2-oxidase (hydroxylating at position 2β – e.g. the conversion of GA₁ into GA₈) appear to be the primary targets of acylcyclohexanediones (Griggs *et al.*, 1991; Nakayama *et al.*, 1991). These findings are supported by analytical data, generally showing that growth reduction is accompanied by lowered levels of biologically active GAs (e.g. GA₁) and their inactive metabolites (e.g. GA₈), but increased concentrations of the inactive immediate (e.g. GA₂₀) and earlier precursors (Nakayama *et al.*, 1991; Adams *et al.*, 1992; Kamiya *et al.*, 1992; Rademacher *et al.*, 1992; Santes and García-Martínez, 1995; Brown *et al.*, 1997; Junttila *et al.*, 1997; Lee *et al.*, 1998; Na *et al.*, 2011). In selected cases, compounds like prohexadione-calcium and trinexapac-ethyl may, paradoxically, lead to increases in shoot growth, most likely by protecting endogenous active GAs from being metabolically inactivated by GA 2-oxidase (Hisamatsu *et al.*, 1998). Likewise, the inactivation of exogenously applied GA₁ by 2β-hydroxylation can be inhibited by simultaneous treatment with an acylcyclohexanedione, resulting in increased GA activity (Nakayama *et al.*, 1990a; Sponsel and Reid, 1992; see also Figure 12.2).

2-Oxoglutarate-dependent dioxygenases catalyse many different reactions in plant metabolism (Farrow and Facchini, 2014). Accordingly, some important side activities of prohexadione-calcium and trinexapac-ethyl have been detected: high dosages of these and other acylcyclohexanediones inhibit the formation of anthocyanins in flowers and other plant organs. It has been suggested that 2-oxoglutarate-dependent dioxygenases, in particular flavanone 3-hydroxylase, which is involved in the biosynthesis of anthocyanidins are targets for these growth retardants (Rademacher *et al.*, 1992). This hypothesis has been confirmed by the finding that young shoots of apple are unable to convert eriodictyol by 3-hydroxylation into flavonoids such as catechin after treatment with prohexadione-calcium. Instead, eriodictyol accumulates and large amounts of luteoliflavan, which does not normally occur in apple tissue, can be found. This shift in flavonoid metabolism is seen as the major underlying reason for reduced susceptibility of treated pome fruit trees to bacterial and fungal diseases: luteoforol, the highly reactive and unstable precursor of luteoliflavan, shows clear *in vitro* biocidal activity against a number of bacterial and fungal pathogens, including *Erwinia amylovora* and *Venturia inaequalis*, the causal agents of fire blight and apple scab, respectively (Spinelli *et al.*, 2005). Apigeninidin, luteolinidin and their derivatives, which are also 3-deoxy flavonoids, act as phytoalexins in *Sorghum bicolor* (Lo *et al.*, 1999). For more information see Römmelt *et al.* (2003), Halbwirth *et al.* (2003), Rademacher (2004b) and Halbwirth *et al.* (2006). Prohexadione-calcium and trinexapac-ethyl reduce ethylene formation in sunflower cell suspensions and in leaf disks of wheat (Grossmann, 1992). Ethylene is generated from aminocyclopropanecarboxylic acid (ACC) in a reaction catalysed by ACC oxidase. This is a dioxygenase that requires ascorbic acid as a cosubstrate, whereas 2-oxoglutaric acid and similar compounds inhibit its activity (Iturriagagoitia-Bueno *et al.*, 1996). Employing an enzyme system

prepared from ripe pear fruits, it could be shown that prohexadione-calcium also inhibits ACC oxidase, presumably by displacing ascorbate from the enzyme's active site (Rademacher *et al.*, 1998).

Long after its introduction, it could also be demonstrated that daminozide interferes with GA biosynthesis. Considering structural similarities between daminozide and 2-oxoglutaric acid and newly interpreting older results from the literature, it was proposed that daminozide, like acylcyclohexanediones, would block GA formation as an inhibitor of 2-oxoglutarate-dependent dioxygenases (Rademacher, 1993). This hypothesis was later proved by working with an enzyme preparation derived from cotyledons of *Phaseolus coccineus* and by analyzing the GAs of treated peanut plants (Brown *et al.*, 1997).

Daminozide

The plant growth-regulatory activity of daminozide was first reported by Riddell *et al.* (1962) and its market introduction by Uniroyal Chemical Co., Inc. followed in 1963. Daminozide was used in several fruit tree species, particularly in apple, to reduce vegetative growth and improve fruit colouration and firmness (trade name: Alar[®]). Treatment of peanut plants led to more compact plants, allowing for a more efficient harvesting (trade name: Kylar[®]). However, due to toxicological concerns, its use on food crops was banned in 1989. The compound is still used as B-Nine[®] in ornamentals, with Chemtura Corp. a major supplier.

Trinexapac-ethyl and prohexadione-calcium

Trinexapac-ethyl was introduced to the scientific community by Kerber *et al.* (1990) and Adams *et al.* (1992). Ciba-Geigy AG (now Syngenta) started with sales in 1992. Prohexadione-calcium originates from Kumiai Chemical Industry Co., Ltd. and Ihara Chemical Industry Co., both of Japan. The first international publication on its properties as a growth retardant was by Nakayama *et al.* (1990a). In 1991, BASF obtained a license to develop this compound in Europe, North America and several other countries. Kumiai launched prohexadione-calcium in Japan as an anti-lodging agent in rice, while BASF started commercialisation of the compound in combination with mepiquat chloride as a stem stabiliser in cereals in 1998 and solo for use in pome fruit trees in 2000.

12.3.4 16,17-Dihydro-gibberellins

16,17-Dihydro-GAs represent the most recent group of growth retardants. A number of different structures of this type, mostly GA₅ derivatives, have been shown to reduce shoot elongation in *Lolium temulentum* (Evans *et al.*, 1994; Mander *et al.*, 1995, 1998) and other grasses (King *et al.*, 1997). Evidence is available that their growth-retarding activity is due to an inhibition of dioxygenases, which catalyse the late stages of GA metabolism, particularly GA

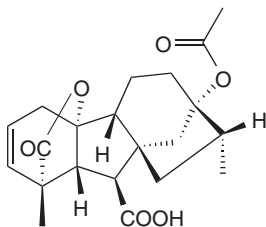


Figure 12.6 Chemical structure of *exo*-16,17-dihydro-GA₅-13-acetate.

3-hydroxylation (Takagi *et al.*, 1994; Foster *et al.*, 1997; Junttila *et al.*, 1997). Treating plants with 16,17-dihydro-GA₅ results, indeed, in changes of GA levels similar to the ones caused by acylcyclohexanediones: In *Lolium temulentum* (Junttila *et al.*, 1997) and in *Sorghum bicolor* (Foster *et al.*, 1997) the levels of GA₁ declined, whereas GA₂₀ accumulated significantly.

With a view to find new anti-lodging compounds for small grains, several 16,17-dihydro-GA₅ derivatives have been systematically tested in suitable formulations. As a result of these investigations, *exo*-16,17-dihydro-GA₅-13-acetate (Figure 12.6) emerged as the most active growth retardant ever known for graminaceous plants. Under greenhouse conditions effects with as little as 500 mg per hectare can be monitored in wheat and barley. However, in order to reduce the risk of lodging under practical conditions, rates in the range of 20 g per hectare have to be used (Rademacher *et al.*, 1999). In order to explain the high biological activity it can be assumed that *exo*-16,17-dihydro-GA₅-13-acetate and related structures compete very effectively in grasses with the natural GA substrates, e.g. GA₂₀, for the respective enzymatic sites (Takagi *et al.*, 1994; King *et al.*, 2004). In contrast to graminaceous plants, *exo*-16,17-dihydro-GA₅-13-acetate and related structures are virtually inactive in reducing shoot growth in any other plant species tested (Rademacher *et al.*, 1999). In spite of the promising results obtained with *exo*-16,17-dihydro-GA₅-13-acetate, its synthesis from GA₃ in bulk quantities proved to be too expensive for commercialisation (Rademacher, unpublished).

12.4 Uses for gibberellins and inhibitors of gibberellin biosynthesis in crop production

Space limitation does not permit coverage of all practical uses that have been established for the different GAs and inhibitors of GA formation. Therefore, only the major uses and some recent additions are referred to. Detailed information on how to use a given PGR in a distinct crop can be found in the respective labels, which are provided by the distributing companies via the internet.

12.4.1 Wheat, barley, rye, oats and other small-grain cereals

The production of wheat and other small grains has undergone drastic changes since the introduction of science-based agricultural methods. This development is particularly obvious in West Europe with its maritime climate, long days at the time of grain filling and other growing conditions favourable for winter wheat. Productivity data are almost continuously available for Germany since 1878. Starting at yield levels of some 1.3 tonnes per hectare, just above 2.0 t/ha was reached prior to World War I and, after a post-war dip, again in the 1930s. However, enormous increases in yield levels could be achieved since the beginning of the 1950s: within six decades, productivity was almost quadrupled from approximately 2.0 to 7.5 t/ha. Similar degrees of intensification were reached in countries with comparable production conditions such as France and the United Kingdom (UK) (FAO-STAT; Rademacher, 2010b). Likewise, seed yield per unit of land could also be raised significantly in other small-grain species such as barley, rye, triticale, oats and spelt. It is estimated by several authors that the increases in productivity have mainly resulted from increased and better-targeted fertilisation (40–45%), followed by breeding (25–30%) and crop protection plus soil management (25–30%) (Sturm *et al.*, 1994). These factors for success are closely interconnected: dispensing, for instance, with fungicides treatments could certainly lead to yield reductions of much more than 30% under adverse production conditions. It must also be noted that the mentioned achievements have been a major prerequisite for creating modern and wealthy societies in industrialised countries with limited area available for agriculture.

With rising production intensity in Germany, UK, France and other countries, lodging became increasingly a problem in cereal cultivation in the 1950s and 1960s: heavy ears could no longer be kept upright by long stems, particularly when their leverage was increased by wind and rain. Lodging occurs mainly during the two months preceding harvest and may drastically reduce profitability through reduced yield and quality and increased costs for harvesting and grain drying (Table 12.1). If lodging occurs early (e.g. shortly after anthesis), its impact on seed yield and quality will be more intense as compared with lodging close to harvesting (Hoffmann, 1992). Under UK growing conditions, severe lodging in cereals may be expected in one out of three to four years (Baker *et al.*, 2014). It is likely that the situation is similar in other countries with high intensities of production. Assuming an average yield of 7.5 t/ha and a producer price of 180.00 €/t for wheat, a reduction in yield of 20% due to lodging is equivalent to 270.00 €/ha. Additional financial losses are likely to result from inferior grain quality and increased costs for harvesting and grain drying. The use of anti-lodging products in wheat has been banned in Sweden in 1987 in order to reduce production intensity and, thereby, lower the negative impacts agriculture may have on the environment. Not least due to this, a reduction of wheat productivity by approximately 25% has resulted in comparison to countries with similar production

Table 12.1 Negative impacts of lodging on wheat yield and quality (typical values after Pinthus, 1973; Anderson, 1979; Jung and Rademacher, 1983; Hoffmann, 1992; Easson *et al.*, 1993; Berry *et al.*, 2004; Baker *et al.*, 2014 and after results of BASF field trials).

Parameter	Effect
Total grain yield	Decreased by 10–40% (up to 80% in extreme cases)
1000-grain-weight	Decreased by 8–15%
Crude protein content of seeds	Relative increase by 3–20%
Carbohydrate content of seeds	Relative decrease by 10–17%
Milling quality	Decreased
Baking quality	Decreased
Presence of mycotoxins	Significantly increased risk
Costs for harvesting	Increased by up to 50%
Costs for grain drying	Increased by 20–30%

conditions such as Denmark, Germany or the UK (data from FAOSTAT). A few years ago, the ban on PGR use in Swedish wheat production was lifted.

Two forms of lodging can be differentiated: (1) stem lodging occurs when heavy wind and rainfall exert a force that breaks the stem base. Often, stem lodging is found after a severe thunderstorm. Eyespot, caused by *Pseudocercospora herpotrichoides*, and other foot rot diseases may intensify the risk of stem lodging. (2) Root lodging is typically observed when, after several days of rainfall, the plant's root system is unable to keep the stem, with its heavy, water-soaked ear, upright. The risk of both forms of lodging is strongly influenced by cultivar and husbandry factors, including sowing date, seed rate, drilling depth and rate of nitrogen application. In spite of this knowledge, the use of anti-lodging products has become an integral part of the production system in order to secure seed yield and quality. These products reduce stem length, thereby lowering the leverage of the ear and other upper plant parts. Increased stability results also from histological changes caused in the stems (Petry *et al.*, 1989). Evidence is also available that anti-lodging agents lead to increases in root growth, thereby providing better anchorage against falling over and enabling plants to absorb water and nutrients more effectively (Rademacher, 2015).

Breeding for short-stawed varieties has only partly contributed to stem stabilisation under production conditions targeted for high yield and quality. It is suggested that the optimum mature height of winter wheat in the UK is close to 80 cm. Shorter stems would have a negative impact on light interception, encourage leaf diseases and make harvesting more difficult (Flintham *et al.*, 1997; Austin, 1999; Berry *et al.*, 2004). Consequently, breeders are relying to a considerable extent on stem shortening 'when needed' by means of PGRs. This is also reflected by the fact that on approximately 34% of the land in Germany devoted to seed propagation in 2015 for wheat, barley, rye, triticale, oats or spelt varieties with a lodging susceptibility rated

from 'medium' to 'very high' (grades 5 to 9) were grown. Even grade 4 varieties ('low to medium'), which are propagated on approximately 42% of the area (Anonymous, 2015b), are candidates for anti-lodging treatments, particularly when grown at high intensity. This situation is similar in the other major cereal-producing countries in Europe.

After its market introduction in 1965, chlormequat chloride was the first PGR to be used on a large scale as an anti-lodging agent in cereal production. Meanwhile, additional PGRs have been introduced for stem stabilisation and lodging control in small grains. Combinations of chlormequat chloride or mepiquat chloride with trinexapac-ethyl or prohexadione-calcium, either by tank-mixing or by using ready-mix formulations, currently represent the best technical solutions for lodging control: chlormequat chloride and mepiquat chloride act at relatively low temperatures and may be used early in the season. Their onset of activity is relatively slow, but long-lasting. Complementing these compounds, trinexapac-ethyl and prohexadione-calcium act comparatively quickly, but are relatively short-lived. Furthermore, they require somewhat higher temperatures for activity. When chlormequat chloride is applied at early stages of tillering, it increases the number of fertile tillers in addition to reducing stem length. This can be of special interest after winter losses of plants. Barley is less responsive to chlormequat chloride and mepiquat chloride than wheat, rye, triticale or oats. Therefore, products containing trinexapac-ethyl and prohexadione-calcium or the ethylene-releasing ethephon are preferentially used to reduce the risk of lodging in this crop. Additional information on lodging in small grains and on the use of anti-lodging agents in cereal production can be found in Easson *et al.* (1993), Berry *et al.* (2004), Rademacher (2010b), Berry and Spink (2012) and Baker *et al.* (2014). Different anti-lodging products are listed in Table 12.2.

Area- and value-wise, stem shortening in small grains (and in intense rice production in Japan and South Korea) to reduce the risk of lodging is the main application of PGRs worldwide. Indeed some 25% of the global PGR sales (equalling approximately € 270 million) are represented by stem stabilisers. The usage of such products is general practice in countries with intense production of wheat, barley, rye, triticale and oats, such as France, Germany and the UK. For instance, 89% of the winter wheat, 76% of the winter barley, 73% of the oats and 100% of the winter rye acreage were treated with anti-lodging products in the UK in 2012. Chlormequat chloride and trinexapac-ethyl rank third and seventh, respectively, in terms of treated area of all crop protectants in all field crops in this country (Garthwaite *et al.*, 2013).

Anti-lodging products for small-grain cereals play only a minor role in countries where these crops are generally grown at relatively low levels of intensity. In the USA, Canada and Australia, climatic factors, in particular unfavourable temperatures and shortage of moisture, represent the main restrictions. Since sufficient arable land is available, significant surpluses for export can still be produced on a national level. However, the use of

Table 12.2 GA₃ and inhibitors of GA biosynthesis used in cereal and rice production.

Crop species	Use	Active ingredient (s)	Main trade name (s)	Main producer(s) and/or supplier(s)
Rice	Acceleration of germination and seedling development	GA ₃	Release [®]	Valent BioSciences
Wheat, Rye, Oats, Triticale	Reduced risk of lodging	Chlormequat chloride	Cycocel [®]	BASF
Wheat		Chlormequat chloride + Imazaquin	Meteor [®]	BASF
Barley, Wheat, Rye, Triticale		Mepiquat chloride + Ethephon	Terpal [®]	BASF
Wheat, Barley, Rye, Oats, Triticale, Spelt		Trinexapac-ethyl Prohexadione-Ca + Mepiquat chloride	Moddus [®] / Medax [®] / Canopy [®]	Syngenta BASF
Rice		Uniconazole-P	Lomica [®]	Sumitomo
		Uniconazole-P in coated fertilizer granules	Rakuichi [®]	Sumitomo
		Paclobutrazol	Smarect [®]	Syngenta
		Prohexadione-Ca	Viviful [®]	Kumiai

PGRs is indispensable when wheat or other small grains are grown more intensively in areas where this is possible (Rademacher, 2010b). To date, chlormequat chloride and ethephon represent only niche markets in Canada. Ethephon has recently been withdrawn, whereas trinexapac-ethyl has just been registered as an anti-lodging agent in the USA. A survey with agronomists working for major rural supply companies across Australian grain-growing regions has recently been conducted: only 20% of the participants recommended the use of PGRs for crop management in wheat (Acuna, 2014). Chlormequat chloride and ethephon are available for cereal lodging control in Australia. The registration of trinexapac-ethyl is expected for 2015.

12.4.2 Rice

In direct-seeded rice, application of GA₃ as a seed dressing is relatively common. This treatment significantly improves germination percentage, seedling

emergence and seedling height and is especially important at sub-optimal temperatures (Dunand, 1992).

As in small grains, lodging can also be a severe problem in intense rice production (Jennings and Sornchai, 1964; Moody, 1986; Nishiyama, 1986; Yoshinaga, 2005; Shibata and Takebayashi, 2007; Salassi *et al.*, 2013). However, modern semi-dwarf cultivars produce relatively high yields, while being largely lodging-resistant. In spite of this option, many farmers still prefer to grow tall but lodging-susceptible varieties, which are tastier and achieve a higher price. For instance, Japanese consumers prefer rice from the traditional long-strawed cultivar 'Koshihikari', which is grown on approximately 35% of the Japanese rice-producing area. Without treatment, 'Koshihikari' typically reaches a final shoot length around 110 cm and is very prone to lodging (Shibata and Takebayashi, 2007). As with cereal grains, several husbandry methods can be employed to minimise lodging in rice. Reducing overgrowth of stem and leaves by applying PGRs at mid-tillering stage reduces lodging incidence and gives a generally improved plant stature (Yim *et al.*, 1997). In 1988 some 12% of the Japanese rice-growing area was treated with stem stabilisers (Schott and Walter, 1991). One may assume that this percentage has at least remained constant.

Whereas stem stabilisers are applied to cereal plants by spraying the leaves, granules for throwing into the paddy field are preferred in rice production, particularly in Japan and South Korea. In order to be absorbed via the roots, such stem stabilisers have to be relatively persistent. Accordingly, preparations based on long-lived uniconazole-P and paclobutrazol are the main active ingredients used as anti-lodging agents in this crop. Prohexadione-calcium, which has to be spray-applied, is only of minor importance (Table 12.2).

12.4.3 Sugarcane

Ethephon, glyphosate or other herbicides are often used as chemical ripeners in sugarcane production. They have to be applied via aircraft or ground-operated booms. By rapidly reducing the sink demand of young and growing plant parts, sucrose storage within the stalk is accelerated leading to high harvest yields. Even better effects can be achieved with trinexapac-ethyl (Resende *et al.*, 2000; Rixon, 2007). Meanwhile, trinexapac-ethyl is registered in Brazil, Australia, the USA and other countries for use in this crop.

12.4.4 Pasture and turf grasses

GA₃ has found some use in the USA and other countries to stimulate shoot growth in pasture grasses (Matthew *et al.*, 2009). However, much more interest is directed towards reducing shoot elongation. Here, inhibitors of GA biosynthesis are important in high-intensity fine turf, particularly on golf courses. A main reason is to reduce vertical leaf growth, which

Table 12.3 PGRs used in pasture and turf grasses.

Crop species	Use	Active ingredient(s)	Main trade name(s)	Main producer(s) and/or supplier(s)
Forage grasses	Stimulation of shoot growth	GA ₃	ProGibb®	Valent BioSciences
Grasses grown for seed	Reduced risk of lodging	Trinexapac-ethyl	Palisade®	Syngenta
	Reduced risk of lodging Suppression of annual bluegrass	Prohexadione-Ca	Apogee®	BASF
Turf grasses	Improved turf quality Less need for mowing Suppression of annual bluegrass	Flurprimidol Paclobutrazol Prohexadione-Ca	Cutless® Trimmit® Regalis®	SePRO Syngenta BASF
	Improved turf quality Less need for mowing	Trinexapac-ethyl	Primo® Maxx	Syngenta

leads to smoother and more uniform playing surfaces. Darker leaf colour, intensified root growth, reduced water consumption, seed head suppression of unwanted annual bluegrass (*Poa annua*), and, not least, less need for mowing are additional benefits. Trinexapac-ethyl, paclobutrazol, flurprimidol and different combinations of these retardants are the main PGRs used for this purpose in the USA. Similar products for growth regulation in fine turf grasses are available in several other countries. Prohexadione-calcium is available as a PGR for use on turf grasses in Germany. A recent survey of growth regulators in turfgrasses is available from March *et al.* (2013).

As in cereal grain production, lodging may also be a problem when grasses are grown for seed production. Trinexapac-ethyl and prohexadione-calcium are the main active ingredients used to reduce this risk, particularly in the US state of Oregon, where grass grown for seed is a major business. Prohexadione-calcium has a small advantage in performance: most likely due to its more immediate action after application (Rademacher, 2014), it inhibits seedhead formation in unwanted *Poa annua* (Beam and Askew, 2005). PGRs used in pasture and turfgrasses are listed in Table 12.3.

Table 12.4 PGRs used in oilseed rape.

Crop species	Use	Active ingredient(s)	Main trade name(s)	Main producer(s) and/or supplier(s)
Oilseed rape	Improved winter survival	Tebuconazole	Folicur®	Bayer
	Improved shoot structure	Metconazole	Caramba®	CropScience BASF
	Reduced risk of lodging	Metconazole + Mepiquat chloride	Caryx®	BASF
	Control of pathogens	Paclobutrazol + Difenconazole	Toprex®	Syngenta
	Improved shoot structure Reduced risk of lodging	Trinexapac-ethyl	Moddus®	Syngenta

12.4.5 Oilseed rape

Winter oilseed rape (*Brassica napus*, *ssp. napus*) has become an important oilseed crop in many European countries and elsewhere. It can be kept from too intensive growth in late autumn, thereby making it less vulnerable to freezing and desiccation in winter. Later in its development, yield losses due to lodging may occur, which can also be reduced by stem-shortening agents (Kightley, 2001; Berry and Spink, 2009; Baker *et al.*, 2014). The leading compounds used are the triazoles tebuconazole and metconazole, which are marketed for this purpose in France, the UK, Germany and several other European countries. Recent introductions are the combination of metconazole with mepiquat chloride and paclobutrazol with the fungicide difenoconazole (Table 12.4). Tebuconazole and metconazole are primarily used as fungicides in a number of crop plants including oilseed rape. Their shoot growth-reducing activity is restricted to oilseed rape and a few other species. Figure 12.7 demonstrates that they produce a pronounced reduction in GA levels in oilseed rape.

12.4.6 Cotton

Cotton is a perennial plant, which, however, is cultivated in most countries in an annual cycle (Edmisten *et al.*, 2010). In its native habitat, cotton plants do not die in the autumn, but continue to grow until environmental conditions become too restrictive. Another growth characteristic associated with its perennial nature is its indeterminate fruiting habit. Rather than

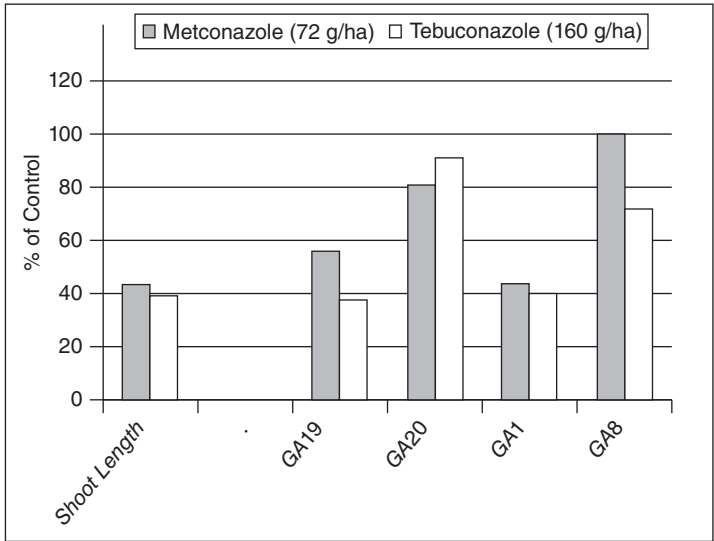


Figure 12.7 Effects of metconazole (applied as Caramba[®]) and tebuconazole (applied as Folicur[®]) on shoot growth and GA levels in glasshouse-grown seedlings of oilseed rape, cv. 'Akela'. Absolute values in control plants: Length of shoot axis 6.3 cm; GA₁₉ 3.9 µg/kg, GA₂₀ 22.7 µg/kg, GA₁ 9.8 µg/kg, GA₈ 5.3 µg/kg of shoot dry weight. Other GAs detected: GA₂₉, GA₃₄, GA₄₄. (S.J. Croker, P. Hedden and W. Rademacher, unpublished)

flowering during a distinct period following vegetative growth, cotton plants simultaneously produce vegetative and fruiting structures. In order to enable high yield and quality formation and to allow efficient mechanical harvesting, intense usage of PGRs has become a standard practice in many cotton-producing countries. The regime typically comprises control of vegetative growth by mepiquat-containing products, defoliation by thidiazuron and boll-opening by ethephon.

The growth-retardant mepiquat chloride was commercially introduced for vegetative growth control in cotton in the USA in 1980 and has since become a cornerstone of modern cotton production (Kerby *et al.*, 1996; Edmisten *et al.*, 2010). Instead of or in addition to mepiquat chloride, chlormequat chloride is used for the same purpose in some countries, for instance in Australia. Another variant is mepiquat pentaborate, which was brought to the US market in 2003 (Stapleton and Via, 2003). Due to its more rapid uptake (Rademacher, unpublished) and the nutritive value of the contained boron, the pentaborate form of mepiquat leads to better plant performance under distinct growing conditions as compared to its chloride salt (e.g. Norton and Borrego, 2006). Mepiquat chloride is also available in combination with kinetin or cyclanilide (Table 12.5). However, in the majority of cases the

Table 12.5 Inhibitors of GA biosynthesis used in cotton production.

Crop species	Use	Active ingredient(s)	Main trade name(s)	Main producer(s) and/or supplier(s)
Cotton	Management of shoot growth	Mepiquat chloride	Pix®	BASF/Arysta LifeScience
	Earlier harvesting	Mepiquat chloride + Kinetin	Mepex® Gin Out	Nufarm
	Improved fibre quality	Mepiquat chloride + Cycilanilide	Stance®	Bayer CropScience
		Mepiquat pentaborate	Pentia®	BASF

different mepiquat-containing products on the market give comparable results (e.g. Jost *et al.*, 2006).

Treatment of cotton plants with mepiquat starting at the beginning of flowering reduces the intensity of new growth and, thereby, improves the sink strength of the first six to ten fruiting branches. This is of great importance because the bolls retained in this part of the plant will give the highest and earliest yields. The shifting of assimilates into the older fruiting structures is at the expense of younger fruits, which the plant is continuously forming, even late in the season and which are unlikely to contribute to yield at mechanical harvesting. Plants treated with mepiquat produce higher yields and can, typically, be harvested three to ten days earlier than untreated plants. Earliness is of great importance because harvesting can often be performed prior to periods of rainfall. This would also reduce the incidence of fungal diseases. Further benefits from short-season production may result from savings in late-season irrigation and insecticide costs. Finally, decreases in quantity and quality of the lint due to weathering are reduced in the oldest, first-opened cotton bolls. Valuable contributions on different aspects of using mepiquat chloride in cotton have been presented by Cathey and Meredith (1988), Kerby *et al.* (1986, 1996), Cook and Kennedy (2000) and Gwathmey and Clement (2010).

12.4.7 Peanuts

The foliage of peanut plants is still green at harvesting such that excessive vine growth may reduce digging efficiency. Prohexadione-calcium, which is registered for this use in the USA, retards vegetative growth and improves

the visibility of rows, resulting in improved harvesting efficiency. Pod yield and kernel quality may also be improved (Jordan *et al.*, 2001, 2008).

12.4.8 Opium poppy

Lodging is also a problem in poppy cultivation. Trials in Tasmania, Australia, where a major portion of the global legal opium poppy production is located, were therefore conducted with trinexapac-ethyl and prohexadione-calcium to overcome this problem. Surprisingly, it was found that treatments with these compounds did not only improve lodging resistance, but also changed the alkaloid spectrum in the harvested plant material in a desirable way: more thebain, which is of higher value, is formed at the expense of lower-valued oripavine (Cotterill, 2005). It has been postulated that a 2-oxoglutarate-dependent dioxygenase, which catalyses the conversion of thebain into oripavine is blocked (Hagel and Facchini, 2010; Dean, 2011). Trinexapac-ethyl is now registered in Australia for use in opium poppy.

12.4.9 Fruit trees growing in temperate climate

Gibberellins and growth retardants have been used for many years in the cultivation of pome and stone fruit trees such as apples, pears, peaches, plums and cherries, which are typically grown in temperate climates. It is much more difficult to apply PGRs in such perennial fruit crops than in annual arable crops. Mistakes made in one year may often lead to problems in the years following. On the other hand and in contrast to field crops, fruits, typically, represent a higher-value crop and, hence, allow the use of more elaborate and expensive products. Recent overviews on different PGR uses in fruit production have been presented by Petracek *et al.* (2003), Greene (2010), Rademacher and Brahm (2010) and Looney and Jackson (2011). Table 12.6 gives an overview of uses for GAs and inhibitors of GA biosynthesis in fruit trees.

Gibberellins

In pears, parthenocarpic fruit formation can be achieved with GA₃. This may lead to an increased fruit set and is particularly important when the generative part of the flower has been damaged by frost or when there has been poor pollination. Some varieties, e.g. 'Williams' and 'Abate Fetel', are more responsive than others. GA₃ may also be used in sweet cherries to produce brighter-coloured, firmer fruits with increased size. The mixture of GA₄ and GA₇ is often used by apple growers to reduce fruit russetting, a superficial disorder in which the fruit surface is interrupted by raised corky outgrowths. Evidence is available that the GA₄ component is the primary active ingredient for the control of russetting, whereas GA₇ rather inhibits flowering and

Table 12.6 GAs and inhibitors of GA biosynthesis used in temperate fruit trees.

Crop species	Use	Active ingredient(s)	Main trade name(s)	Main producer(s) and/or supplier(s)
Apples	Reduction of russetting	GA _{4/7}	Regulex®	Syngenta
			ProVide®	Valent BioSciences
	Improved fruit shape and size	GA ₄ (only traces of GA ₇)	Novagib®	Fine Agrochemicals
		GA _{4/7} + 6-benzyladenine	Promalin®	Valent BioSciences
	Control of shoot growth	Paclobutrazol	Cultar®	Syngenta
	Control of shoot growth	Prohexadione-Ca	Regalis®/ Apogee®	BASF
	Improved fruit set and yield Control of fire blight			
Pears	Parthenocarpic fruit set	GA ₃ and GA _{4/7}	Berelex® and Regulex®	Syngenta
			Gibb 3 and Gibb Plus	Globachem
	Control of shoot growth	Prohexadione-Ca	Regalis®	BASF
	Improved fruit set			
	Control of fire blight			
Peaches, Nectarines, Plums	Control of shoot growth	Paclobutrazol	Cultar®	Syngenta
	Control of shoot growth	Paclobutrazol	Cultar®	Syngenta
Sweet cherries	Improved fruit quality	GA ₃	ProGibb®	Valent BioSciences
	Control of shoot growth	Prohexadione-Ca	Apogee®	BASF

reduces return bloom (Carlson and Croveti, 1990). Hence, preparations low in GA₇ may have an advantage for this use. Combined with the cytokinin benzyladenine, GA_{4/7} is also used to improve size and shape of apples fruits. Fruit elongation and development of more prominent calyx lobes in Red Delicious apples are of special interest in North America.

Inhibitors of gibberellin biosynthesis

Proper tree growth management is of major concern in commercial pome fruit production: avoiding excessive shoot growth will induce earlier flowering and fruiting in young trees. Older trees have to be restricted to their allocated space, thereby reducing crowding and shading. The crowns of fruit trees should be sufficiently open to allow good light penetration to the inner parts of the canopy, thereby improving photoproductivity and fruit colouration. Additionally, efficient crop protection is significantly facilitated in such trees. Since the beginning of professional apple and pear production, several techniques have been employed to avoid excessive shoot growth: different types of dwarfing rootstocks and scions have become available, particularly for apple. Different cultivars may also show significant differences in shoot vigour. Dormant and summer pruning are the main cultural practices for shoot control in addition to regulation of fruit set. Other methods include: root pruning, root restriction, stem girdling or stem sawing, limb bending, breaking or wounding, and restrictive fertilisation and irrigation. However, each of these methods is cost-intensive and/or bears a high risk of failure. Furthermore, part of the trees' assimilates or potential assimilates are lost.

Chemical regulation of shoot growth has been practiced over many years by using distinct inhibitors of GA biosynthesis. However, health concerns about daminozide have led to the ban of this compound in edible crops. Additional negative attitudes towards using PGRs for shoot growth regulation resulted from unacceptably high residues of chlormequat in pear fruits, due to excessive use in this crop. In essence, only paclobutrazol remained registered in some countries at the end of the 1990s as a regulator of shoot growth in pome and stone fruits. However, this compound is extremely persistent. Its half-life in the plant and in the soil is in the range of six months. Application is via spraying or as a soil drench. In order to avoid effects on following crops, the label for the UK recommends to withhold using paclobutrazol for up to seven years if an orchard is due for grubbing. Consequently, paclobutrazol is currently registered for use in fruit trees in the UK and Spain as the only European countries. Likewise, there is no legal use of this compound in fruit trees in the US or in Canada.

A new option for controlling growth of fruit trees became available with the introduction of prohexadione-calcium, which has a half-life in plants of approximately 10 to 14 days and of less than one day in microbially active soil. Due to its simultaneous effects on the formation of GAs, ethylene and flavonoids, treated pome fruit trees benefit in several ways (Rademacher *et al.*, 1998; Byers and Yoder, 1999; Greene, 1999; Owens and Stover, 1999; Unrath, 1999; Yoder *et al.*, 1999; Basak and Rademacher, 2000; Rademacher and Kober, 2003; Rademacher *et al.*, 2004; Costa *et al.*, 2006; Rademacher *et al.*, 2006):

- Figure 12.8 demonstrates that GA₁₉ and GA₂₀, inactive precursors of GA₁, accumulate in apple shoots after application of prohexadione-calcium. In contrast, levels of GA₁, the main active GA in apple shoots, decline. The

result is reduced shoot elongation. The labour needed for dormant and summer pruning in treated trees is typically reduced by approximately 30%. Furthermore, a more open canopy allows better light penetration into the central parts of the crown leading to improved fruit quality. Spray application of crop protectants is also made more efficient.

- The inhibition of ethylene formation may be employed to increase fruit set. Since assimilates no longer needed for shoot growth are available, fruit size and internal quality, as well as return bloom do not suffer, provided that over-cropping is avoided by appropriate dosaging and timing.
- Blocking flavanone 3-hydroxylase activity in pome fruit shoots leads to a *de novo* formation of 3-deoxyflavonoids, such as luteoforol and luteoliflavan (Halbwirth *et al.*, 2003; Römmelt *et al.*, 2003; Rademacher, 2004b). Luteoforol shows biocidal activity against several bacterial and fungal pathogens, including *Erwinia amylovora* and *Venturia inaequalis*, the causal agents of fire blight and apple scab, respectively (Spinelli *et al.*, 2005). The triggering of such phytoalexin-like compounds explains why treated pome fruit trees are significantly less affected by a number of diseases. This induction of defence is of particular value to control shoot fire blight, a disease caused by the bacterium *Erwinia amylovora*, which is primarily controlled by using antibiotics, a treatment that is highly controversial (Stockwell, 2014). The action of fungicides is promoted by this enhanced resistance, but also by the more open canopy resulting from reduced levels of active GAs.
- Apple and pear trees treated with prohexadione-calcium are also less affected by insect pests, such as aphids, psyllids and leafhoppers (Brisset *et al.*, 2005; Paulson *et al.*, 2005; Leahy *et al.*, 2006), which synergises the action of insecticides. The underlying biochemical mode of action could also be changes in the spectrum of flavonoids, which may, for instance, repel sucking and chewing insects. However, paclobutrazol-treated trees are also less affected by insect pests (Campbell *et al.*, 1989). This indicates that a thicker epidermis or thicker cell walls resulting from reduced elongation growth may also be the reason for less insect attack.

12.4.10 Fruit and nut trees growing in subtropical and tropical climates

Uses of GA₃ and growth retardants in fruit and nut trees growing in warm climates are listed in Table 12.7. GA₃ is of major interest in most citrus-growing countries for use with a variety of citrus species and varieties (El-Otmani *et al.*, 2000; Coggins and Lovatt, 2014). It is mainly applied to increase fruit set, delay harvesting and improve fruit quality. In Navel oranges, rind aging may be delayed. Growers can spray part of their groves to allow sequential harvesting after picking fruits from non-treated blocks early. A delayed harvest may also be used to 'store' citrus fruits (which are non-climacteric) on the tree

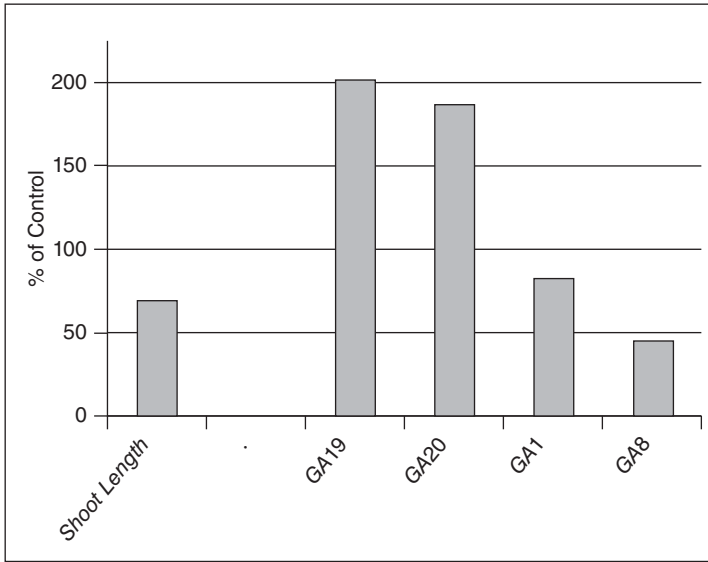


Figure 12.8 Effect of 25 g/ha of prohexadione-calcium (applied as Regalis®) on shoot growth and GA levels in glasshouse-grown plantlets of apple, cv. 'Jonagold' on rootstock M9. Absolute values in control plants: Growth increment of shoot after treatment 36 cm; GA₁₉ 30.6 µg/kg, GA₂₀ 10.6 µg/kg, GA₁ 4.0 µg/kg, GA₈ 7.8 µg/kg of shoot dry weight. GA₂₉ was also detected. (After Croker *et al.*, 2001)

until a better market window opens. Fruits from treated trees will also display less rind disorders (e.g. rind staining, water spotting, puffy rind, sticky rind). 'Creasing' or 'puff and crease' is an important rind disorder, which is of particular concern in Navel and Valencia oranges and in Satsuma mandarins. The disorder occurs when the rind tissue (orange-coloured flavedo layer plus epidermis) continues to stretch when the albedo layer (white tissue under the rind) has stopped growing. As a result, some parts of the fruit surface appear inflated ('puffy'), whereas other areas are indented (creased). Puffiness occurs also in grapefruits. Application of GA₃, together with certain cultural practices, is employed to reduce the incidence of this disorder. GA₃ may also be used to increase fruit set and yield in Navel and Valencia oranges, as well as in clementines, tangelos and tangerines.

In several countries (e.g. Australia, South Africa, India and Mexico), paclobutrazol and uniconazole or uniconazole-P are used to control excessive shoot growth in fruit and nut trees, such as avocado, mango, litchi, pecan and macadamia. This facilitates tree management (e.g. pruning, application of crop protectants, fruit picking) and more trees may be grown per unit area. Increases in fruit yield and quality are often observed. The longevity of the compounds can be seen as an advantage since less persistent compounds may be degraded too rapidly under the given climatic conditions.

Table 12.7 Uses of GA₃, uniconazole-P and paclobutrazol on sub-tropical and tropical fruit and nut trees.

Crop species	Use	Active ingredient(s)	Main trade name(s)	Main producer(s) and/or supplier(s)
Citrus	Increased fruit set and yield Delay of fruit maturation Improved fruit quality	GA ₃	ProGibb®	Valent BioSciences
Avocados	Control of shoot growth Improved fruit shape and size	Uniconazole-P Paclobutrazol	Sunny® Cultar®	Sumitomo Syngenta
Mangos, Litchis Pecan and Macadamia nuts	Control of shoot growth			

Application of paclobutrazol and uniconazole-P is either via spraying the foliage or as a soil drench. As with paclobutrazol in pome and stone fruits, accurate timing and dosage are important. It is recommended not to carry out soil drenches for the last three years before grubbing an orchard in order to avoid growth retardation in subsequently planted crops. Background information on this type of PGR use is given by Yeshtela *et al.* (2004), Menzel and Le Lagadec (2014) and Pires and Yamanishi (2014).

12.4.11 Grapevines

Testing of GA₃ on wine and table grapes started in the late 1950s (Weaver, 1958; Weaver *et al.*, 1962) and led to the first major practical uses of this new plant hormone. As far as can be judged, the main use of GA₃ on a global scale is still in grapevines, particularly in seedless table grapes, where its use has become a standard practice. Table grapes without seeds are attractive to consumers. However, their small size represents a problem for commercialisation. GA₃, applied at the right time and dosage for a given variety, is used to overcome this problem. In general, treatment at approximately 20 mm of cluster length may be used to 'stretch' the rachis, application at 30% to 80% cap (calyptra) fall reduces berry set and later treatments increase berry size. Under advanced production conditions (e.g. in California, Italy or Chile), seedless varieties may be grown on more than 80% of the area where they

are virtually all treated with GA₃. For additional reading see Dokoozlian and Peacock (2001) and Casanova *et al.* (2009).

Several varieties of (seeded) wine grapes tend to form very dense clusters. At veraison, the pressure exerted by the ripening and expanding adjacent berries causes wounding and leakage of juice, which may lead to bunch rot (caused by *Botrytis cinerea*) and also to sour rot (caused by different bacteria and yeasts). This is of particular concern to vintners, when rainfall keeps the clusters moist, thereby facilitating the spreading of diseases. GA₃ is used to elongate the rachis and reduce berry set. The window for application lies between a cluster length of approximately 7.5 cm and the end of flower opening. Earlier treatments lead primarily to a stretching of the inflorescence, whereas later timings cause berry thinning or the formation of non-seeded shot berries. Berry abortion may result from too high GA levels, a combination of endogenous hormone produced by the developing seeds and that applied externally. Many varieties, for instance of the Pinot family and Chardonnay, respond relatively well to treatment with GA₃. However, Riesling, Sauvignon Blanc and other varieties suffer from poor induction of shoot and flower buds for the next season and, therefore, are not suitable for GA₃ treatment (Petgen, 2005; Molitor *et al.*, 2012). Recently, an alternative strategy was developed through the application of prohexadione-calcium. Trials at BASF in Germany with a view to controlling excessive shoot growth were without success since too high dosages were required. However, in the course of these investigations, an interesting effect on berry-thinning was observed after applying moderate dosages early in the season. This phenomenon was systematically pursued and prohexadione-calcium is now available for this use in Germany and Austria. Independently, Lo Giudice *et al.* (2003, 2004) at Virginia State University also observed that prohexadione-calcium reduced berry set and suggested that this could be of practical interest. However, there was no follow-up in the USA. Mid flower opening, when the caps on 20% to 80% of the flowers have abscised, is recommended as an ideal timing for prohexadione-calcium application, which overlaps with the time window for GA₃. At first glance, it may appear paradoxical that an inhibitor of GA biosynthesis is giving effects equivalent to those of an active GA. First attempts to find out the underlying mechanisms remained inconclusive (Böll *et al.*, 2009). However, it appears likely that prohexadione-calcium blocks the inactivation of active GAs present at the time of treatment. Its continued activity is likely to be the cause of berry thinning. Different from applying the relatively persistent GA₃, this effect will be relatively short-lived and will not cause negative effects for the following season. This explanation corresponds well with the detailed analytical data on the presence of different GAs in developing grape berries (Giacomelli *et al.*, 2013): GA₁ and GA₄, which are both biologically active, give a clear peak at anthesis and decline sharply

Table 12.8 Uses of GA₃, prohexadione-calcium and mepiquat chloride on grapevines.

Crop Species	Use	Active ingredient(s)	Main trade name(s)	Main producer(s) and/or supplier(s)
Seedless table grapes	Cluster stretching Berry enlargement	GA ₃	Activol®	Valent BioSciences
Seeded wine grapes	Cluster stretching	GA ₃	ProGibb®	
	Berry thinning Berry thinning	Prohexadione-Ca	Regalis®	BASF
Table and wine grapes	Control of shoot growth	Mepiquat chloride	Pix® Frastar®	BASF Nisso

thereafter. Prohexadione-calcium is likely to inhibit the hydroxylation of these GAs via GA 2-oxidase into inactive GA₈ and GA₃₄, respectively. GA-like effects resulting from treatment with prohexadione-calcium has been reported for *Matthiola incana*. The authors suggest that inactivation of existing GAs by GA 2-oxidase is blocked by prohexadione-calcium as the underlying mechanism (Hisamatsu *et al.*, 1998).

Control of vegetative growth is another objective in the production of table and wine grapes. Excessive cane growth is particularly observed in warmer climates, when there is ample supply of water. As a result, shading and insufficient air circulation often lead to reductions in yield and quality. Additionally, berry production will severely suffer from more intense competition for assimilates from shoot growth. Mepiquat chloride is in use for vegetative growth control, for instance, in Spain, Japan and South Korea (Lim *et al.*, 2004); chlormequat chloride is used in India. An overview on the mentioned compounds and their uses is given in Table 12.8.

12.4.12 Ornamentals

GA₃ has found some use in the production of ornamentals, when longer stems or peduncles are desirable. Increased flowering is also induced by GA₃ in certain species. However, reducing shoot elongation and promoting lateral branching and flowering in ornamental and bedding plants is of much greater relevance: compact and dense plants require less space in a greenhouse, they need less water for irrigation, they have an increased shelf life, but, above all, they sell better because of their dark green leaves,

which is generally associated with better quality. A wide assortment of growth retardants is currently available to ornamental growers in the USA. These products are based on chlormequat chloride, daminozide, ancymidol, flurprimidol, paclobutrazol and uniconazole-P (Whipker and Latimer, 2013). Detailed use recommendations for a large number of ornamental species and bedding plants raised under greenhouse conditions are given by Whipker (2013). The spectrum of active ingredients available in EU member countries consists primarily of chlormequat chloride, paclobutrazol and daminozide. In Germany metconazole and prohexadione-calcium are additionally allowed for use in ornamentals. The latter compound should not be used in plants with red or blue flowers because of its interference with anthocyanin formation.

Inhibitors of GA biosynthesis, in particular flurprimidol and paclobutrazol, are also being used in the USA and several other countries to reduce the growth of woody and non-woody ornamentals in gardens or parks. Paclobutrazol and flurprimidol often serve as tools to arborists to limit the size and growth of trees and shrubs in power line and utility rights-of-way corridors. Tree growth regulation is regularly applied in high visibility locations such as parks, historic downtowns, residential areas and other places, where trees have a cultural value and where pruning and trimming is difficult to conduct or unwanted (Chaney, 2005). Whereas paclobutrazol is applied via soil injection or soil drenching, flurprimidol is typically administered via stem injection. Table 12.9 gives an overview on uses of GA₃ and different inhibitors of GA biosynthesis in ornamentals.

Table 12.9 GA₃ and inhibitors of GA biosynthesis used in ornamentals.

Crop species	Use	Active ingredient(s)	Main trade name(s)	Main producer(s) and/or supplier(s)
Ornamentals	Promotion of shoot growth Longer stems or peduncles Intensified flowering	GA ₃	ProGibb®	Valent BioSciences
Bedding plants	More compact shoots	Chlormequat chloride	Cycocel®	BASF/OHP
Flowering and foliage plants	Leaves with darker green	Daminozide	B-Nine®	OHP
Bulb plants	Less space required for production	Ancymidol	A-Rest®	SePRO
Woody and non-woody landscape ornamentals		Flurprimidol	Topflor®	SePRO
		Paclobutrazol	Bonzi®	Syngenta
		Uniconazole-P	Sumagic®	Sumitomo
		Metconazole	Caramba®	BASF
		Prohexadione-Ca	Regalis®	BASF

12.4.13 Hybrid seed production

Hybrid cultivars have become common in maize and several other species. However, no cost-efficient hybrid seed production systems exist so far for a number of other crop plants, including wheat. A prerequisite for hybrid breeding is tight pollination control, which avoids self-fertilisation and provides viable pollen from the 'male' plant at the right time and at the right place to fertilise the 'female' plant. GA₃ is used by breeders in several plant species to coordinate the development of the 'male' (fertile) and the 'female' (male sterile) plant for crossing. For instance in rice, GA₃ increases the emergence of the 'female' panicles from the leaf sheath, thereby improving the ability to accept pollen from the 'male' plant (Jagadeeswari *et al.*, 2004; Gavino *et al.*, 2008).

12.5 Outlook

GAs and inhibitors of GA biosynthesis are well established in agriculture, horticulture and viticulture. For the time being, the existing products are suitable for the applications for which they were developed. Therefore and because of the enormous costs involved, it will be difficult to introduce new active ingredients. However, it is likely that additional markets or additional uses for the known PGRs will be found. For instance, climate changes in several European countries have significantly raised the risk of over-growth in winter cereals in late autumn. Therefore, it appears overdue that autumn applications of anti-lodging products are registered in order to avoid winter damage. Prohexadione-calcium is well suited to assist in the production of high-quality strawberry transplants (Reekie *et al.*, 2007). In the same crop, it may also be used to reduce runner formation under long-day conditions, thereby enhancing flower induction and berry yield in the following year (Hytönen *et al.*, 2008). Introducing new combinations of registered PGRs may also offer new and improved solutions. For instance, prohexadione-calcium plus trinexapac-ethyl combine immediate and longer-lasting actions, respectively, in graminaceous species (Rademacher and Strobel, 2010; Rademacher, 2014). Also, active ingredients may lose registration due to toxicological concerns, in which case it is likely that substitutes will be needed. Furthermore, it is possible that a competitive synthesis can be found for *exo*-16,17-dihydro-GA₅-13-acetate. Because of its high specificity, this compound might represent a compound with 'ultra-safe' toxicological features.

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