Invited lecture

BIOREGULATORS APPLICATION IN PEAR PRODUCTION

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Abstract. This short review report the results obtained with the main plant growth regulators/plant bioregulators (PGRs or PBRs) that are currently tested or used as registered pre-harvest and post-harvest compounds. The PBRs used in pre-harvest concern the regulation of fruit setting, bearing, fruit thinning and of shoot growth control. The auxin-like compounds are mainly used for fruit set and for pre-harvest drop control; Gibberellins for fruit set; auxins and citokinins for fruit thinning/quality improvement and prohexadione-Ca (Regalis) for shoot growth and fire-blight control. The PBRs for improving fruit quality and storability can be used in pre- and post-harvest. Aminoethoxyvinylglycine (AVG) is used in pre-harvest while 1- methylcyclopropene (1-MCP) can be used in cold storage room or, in some countries, also in pre-harvest (Harvista) to affect fruit post-harvest maturation and storability management.

Keywords: Auxin-like, GAs, CK, Prohexadione-Ca, AVG, 1-MCP, fruit set, *Pyrus communis.*

Introduction

This review aims to consider the growth regulators (PGRs or PBRs) used currently in the standard cultural management in commercial pear production or under experimental test in the main pear growing areas. PGRs or PBRs refers to natural and synthetic compounds that are used to control vegetative and reproductive tree growth and development. Many of the growth regulators are hormone-like substances that promote, inhibit or affect biological processes in plants. The PBRs must be used considering their efficiency for specific objective as well as their potential environmental impact to improve their performance, preserving human health and productive areas. The PBRs use must be integrated when the appropriate genetic and agronomic choices have been done to contribute increasing or maintaining the sustainability of the considered fruit-tree system. It has to be considered that PBRs are normally enhancing the natural response of the plant. As a consequence, when inappropriate agronomic or genetic choices have been done, it can be risky to rely on the use of PBRs to solve the problems. PBRs normally are active at physiological concentrations and undesirable side effects have always to be taken into consideration, especially when high rate are used.

PBRs function and mimic the activity governed by naturally occurring plant hormones that are generally molecules that exist at low concentration facilitating intercellular communications (Cleland, 1999).

Plant hormones are normally grouped into 5 categories: auxins, gibberellins, cytokinins, abscisic acid and ethylene. Other molecules, classified as growth substances, currently thought to have plant hormones characteristics, include oligosaccharides, jasmonates, salicylic acid, polyamines and brassinosteroids.
Endogenous plant hormones influence different developmental processes in a positive or negative manner but in order to reliably affect some physiological aspects of plants a better understanding of the mechanism of action of the PBRs is still to be further studied. Growth and reproductive potential of the tree can be manipulated using different compounds.

PBRs have the peculiarity that in some cases the same active ingredient can induce different responses (i.e. flower & fruit abscission; flowering) depending on the application time and rate used. In fact, their efficacy is influenced by internal and external factors (cultivar, tree vigor and fruit load, climatic conditions, cultural management, etc.). A holistic approach to understand and comprehend the results obtained with the use of growth regulators should be made considering the cultural management adopted (i.e. nutrition, pruning, training systems, etc.), the physiological status of the plant, the history of the orchard (alternate bearing), and weather conditions during the period of the PBRs application. In this way, models should be developed to forecast and help explaining tree responses.

In pear, the main registered pre-harvest and post-harvest PBRs are auxin-like compounds for fruit set and for pre-harvest drop control; Gibberellins for fruit set; auxins and citokinins for fruit thinning; prohexadione-Ca (Regalis) for shoot growth and fire-blight control; aminoethoxyvinylglycine (AVG), 1-Methylcyclopropene (1-MCP) for pre- and post harvest maturation and storability management.

Fruit setting & fruiting

Fruit setting and fruiting performance both in terms of quantity and quality can be directly affected by Auxin-like, GAs and CK or by the use of anti-gibberellin formulates, that controlling shoot growth, can divert the assimilates versus the fruiting (Fig.1).

Auxin-like substances

Indoleacetic acid (IAA) was found in plants, although auxin-like compounds used in agriculture (PBRs) include synthesized auxin-type hormone as 1-naphthaleneacetic acid (NAA), and its amide (NAAm) that have structural similarities with IAA, but are not found in plants.

Quite interesting to underline that auxin have the peculiarity, when applied at different phenological stages, to lead to effect completely different: in fact the use of

auxin-like formulates during blooming increase fruit set, while when applied at fruitlet stage can induce fruit thinning or before harvest time can reduce the pre-harvest fruit abscission. In fact, NAA applied before expected harvest at the concentration from 100 to 200 g/ha might control pre-harvest drop. A possible explanation of this phenomenon might be related to the fact that fruit abscission is directly related to the expression of genes activating enzymes (i.e., cellulose and polygalacturonase) linked to an increase in ethylene associated with cell wall degradation in the abscission zone. The cell wall degradation enzymes are triggered by auxin reduction to a certain threshold that is normally associated to an ethylene synthesis increase. As a consequence, the auxin/ethylene relationship might control fruit abscission.

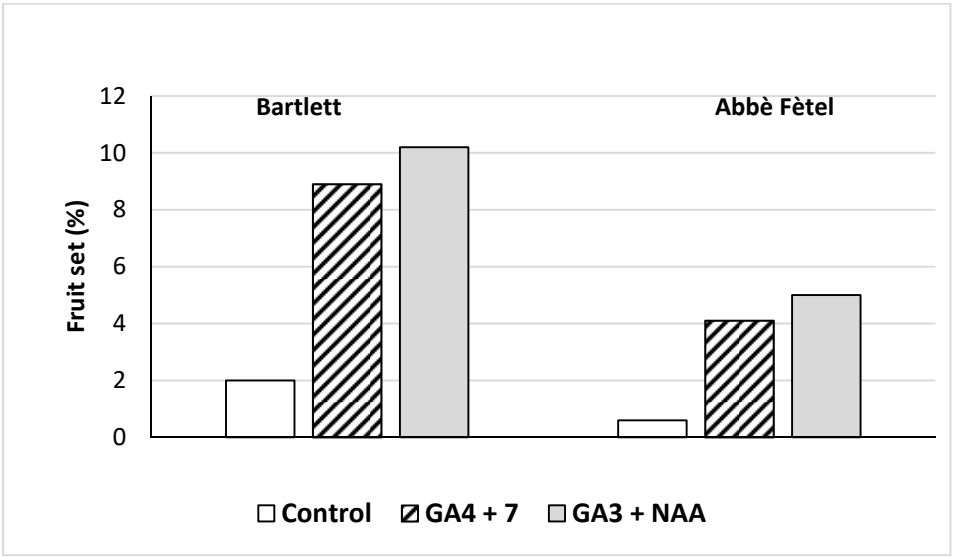

Figure1. Fruit set as affected by GAs and NAA in two pear cultivars (adapted from Sansavini *et al.*, 1981)

GAs and CK

Numerous gibberellins (GAs) are found in plants. GAs are a large group (more than 100) that have some biological activities and share the gibbane ring structure. GAs (both GA3 and GA 4+7 formulates) are used as a setting agent on some cultivar in particular and in situation where fruit set is presumed to be low when spring frost might have compromise fruit set.

Several researches were carried out since the ’60-’70 pointing out the possibility to increase fruit set with the GAs use (Luckwill, 1960; Varga, 1969; Gil *et al*., 1972; Wertheim, 1985; Dussi 2011).

GA4+7 is used alone or in combination with Citokinin (CK). Natural CK are a series of adenine molecules modified by the addition of 5-carbon side chains off the 6th position. Two CK groups include the zeatin-related and adenine-related molecules. CK-like include kinetin and 6-BA. The combination of GA and 6-BA is found in several commercial chemical (Promalin, Progerbalin LG®, Perlan®) belonging to different chemical companies.

The research trials performed on the use of these PBRs started in the ’60 and the ’70, but these PBRs are still used nowadays to increase fruit set and control fruiting.

The applications of auxin during the blooming period at concentration variable as related to the used compound have been able to increase up to 2-3 folds the fruit set percentage as compared to control (Sansavini *et al*., 1981; Nicotra, 1982).

Similar results were obtained with the use of GA3 at 20-30ppm applied at full bloom (fruit set 3 folds higher than untreated control). The effect was pronounced in the varieties that produce parthenocarpic fruits (Conference). It has to be considered that although parthenocarpic fruits might allow achieving an important yield, undesirable side effects as misshapen fruits might occur (Herrero, 1989; Vercammen *et al.,* 2015).

The application of the GA4+7 and 6-BA was initially used for enhancing the length of the fruits in apple but is become an important formulate adopted for fruit russeting control of apple and pear (Looney *et al.,* 2012). Research results indicated that the 6-benziladenine (6-BA) was inducing higher fruit size than control (Deckers and Schoofs, 2002; Stern, 2008; Brighenti *et al.,* 2010).

Some practical examples of the practical use of PBRs to enhance fruit set still refer to Auxins, GAs and CK:

- Auxin (i.e. commercial formulate AF96, a.i. 3,6% di NAA + 0,4% NAA) on William, Comice and Conference is applied at 20% open flower in a single application at a concentration ranging from 800 to 1200 ml/ha.
- AF96 is applied on Abbé Fètel at the same concentration but in two split applications; the 1st at 10-20% Open flower and the 2nd at 50% open flowers.
- Another application on Abbé Fètel concern the combination of AF96 at the concentration of 1000 ml/ha at “white finger bud” stage followed by two applications of GA4+7 and 6-BA (Promalin, Novagib or similar compounds), the 1st at 20% open flower and the 2nd after 4-5 days at a concentration of 250 ml/ha each application.

  As far as GAs alone, the standard application concern the use of GA3 or GA4+7:
- GA3 (several commercial compounds are available, i.e. Berelex at 10%, Falgro or 40%; Gibrelin at 2% or 20% a.i., etc) can be used at a concentration of 1000ml/ha in a single application or in two split application during bloom.
- GA 4+7 (several commercial compounds are available as Regulex10 SG, Gerlagib at 1% a.i., etc) can be applied in a single application at 30-40%

open flower at concentration of 800-1000 ml/ha or in two applications at 30% open flower and 4 days after at a concentration of 400-500 l/ha.

Both auxin-like, GAs and 6-BA have shown to be effective in controlling fruit set in several pear cultivars although there are positive and negative side-effect that have to be carefully considered. For instance it has to be considered that when GAs are used there is a potential risk to negatively affect the return bloom, especially when repeated application are performed to overcome the risk of poor setting compromised by spring frost. When the combination of GA4+7 and 6-BA are applied, the concentration had to be carefully considered since important concentration of 6-BA applied at fruitlet stage could induce fruit abscission.

Fruit thinning

Fruit thinning in pear represent an issue only for some cultivars. In fact, in some years, Bartlett and Conference might produce a high number of fruit that do not reach a marketable fruit at harvest. Conference is per sure the more demanding, but other cultivar might take advantages from the fruit thinning operation. Alternative to the hand thinning have been tested, normally transferring the information and the chemical tested on apple on pears (Williams and Edgerton, 1981; Stan *et al*., 1984; Wertheim, 2000).

In fact, NAA and NAAm have proved to reduce fruit set when applied at 15 to 20ppm approximately 15 to 20 days after full bloom (Williams and Edgerton, 1981, Wertheim, 2000).

Auxin and 6-BA are the PBRs normally used for pear fruit thinning (Giménez *et al*., 2010). A tank mix application of 6-benzyladenine (6-BA) and 1-naphtalene acetic acid (NAA) showed to be, in some situation, very effective in inducing fruit abscission. However concentrations and time of application are essential in obtaining the desired results. Application of NAA and 6-BA induce over-thinning in ‘Conference’ pears when the application was performed at 8 mm fruit diameter, while later applications (15 mm fruit diameter) lead to better results (Maas *et al*., 2010; Fernandes, 2015). Similar effects were obtained in Spain on Conference and Blanquilla cultivar, when a 6-BA + NAA combination was tested (Asin *et al*., 2010). Fruit quality can also be improved by the fruit thinning technique: Canli and Pektas (2015) reported positive effect on fruit quality (expressed as fruit size, weight, diameter and length) when a tank-mix of 6-BA + GA (at 25 and 50 ppm) and of 6-BA at 100ppm were applied. In addition pear fruits reached a higher soluble solids content than the untreated. Results concerning a fruit size enhancement induced by with 6-BA applications were also reported on ‘Williams’ pear (Dussi and Sugar, 2011) and on Conference pear (Basak *et al*., 2016) (Fig. 2).

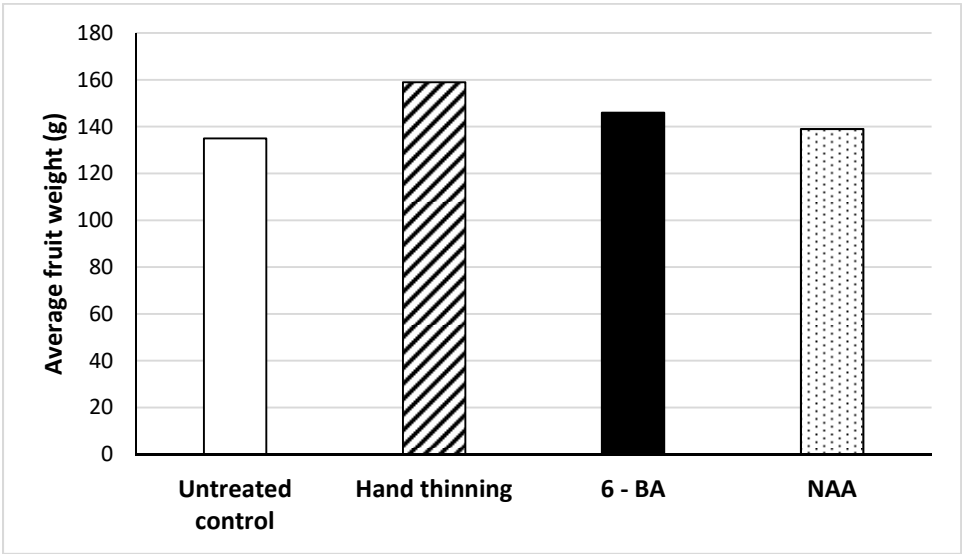

Figure 2. Average fruit weight reached in cv Conference 6-BA and NAA treated trees as compared to control (Source: adapted from Basak et al., 2016).

Other compounds are used on pear for inducing fruit thinning: Ammonium thiosulphate (ATS) showed an efficacy although bloom application increased some undesirable fruit russeting.

More recent interesting fruit thinning results on pear have also been obtained with Metamitrom (commercial name Brevis) (Maas and van der Steeg, 2011) while other experimental PBRs, like abscisic acid (ABA) and 1-aminocyclopropene-1-carboxylic acid (ACC), are also under testing for evaluating their potential thinning efficacy.

In the practice, common strategy are based on the use of NAAm (suggested for Bartlett and Conference at 10 to 50 ppm at petal fall or within 5 to 7 days after petal fall) or NAA (Dirager 3,3% a.i. or Obsthormon 24A at 7,5% a.i.) on Conference and Bartlett at 9mm fruit diameter.

Vegetative growth control

The vegetative control is essential in pear orchard management to select the best wood for the next year production, for return bloom and in general for reducing the competition of the vegetative sinks versus the reproductive ones. The standard method is summer pruning performed by hand. The hand operation is costly and time demanding and alternative chemical and mechanical methods have been tested. The chemical method is based on the use of growth retardants. Several growth retardant have been available until mid-‘80, like Alar, Cicocel, PP333 (Pfammattar, 1977; Modlibowska, and Wickenden, 1977; Nicotra, 1982). Most of these chemicals have

been banned (Alar) or retired (CCC) from the market on pear. PP333 (a.i. paclobutrazol; commercial name Cultar) has not been used in Italy until two years ago, when the authorization procedure for introducing Paclobutrazol was requested and completed and now the growth retardant is available on pear orchard (Fig.3).

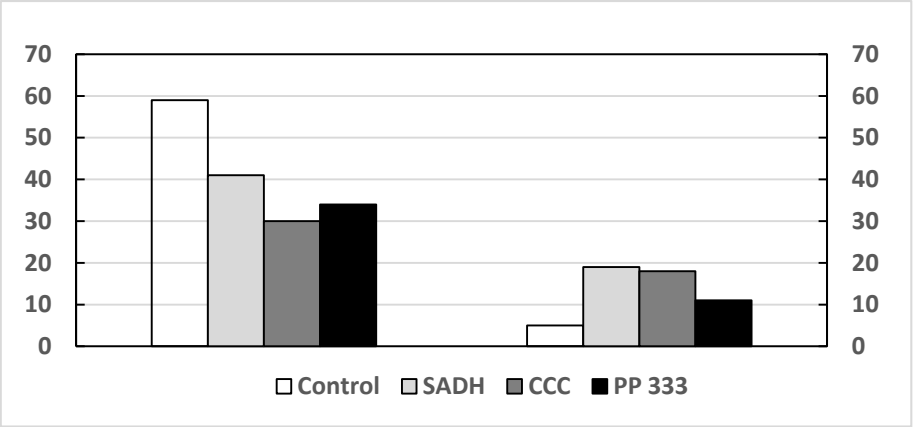

Figure 3. Shoot growth and yield/tree as affected by different growth retardants in AbbèFetel.

Prohexadione-Ca, instead is one of the newest and most interesting growth retardant authorized for apple and pear. It is a shoot growth retardant that may result in different responses depending on the pear cultivar and rate used. An important research activity was carried out with Prohexadione-Ca on pear few years ago (Costa *et al*., 2004; Rademacher, 2004).

The interest on the use of prohexadione-Ca depended upon the need to control vegetative growth in a new high density planted orchard, by the lack of growth retardant available (Alar and CCC) and by the fire blight suppression induced by Prohexadione-Ca. Prohexadione-Ca was selected because represented at that time a new generation of growth retardants characterized by low toxicity. In addition, the mechanism of action was deeply investigated showing its involvement in GAs biosynthesis and its interesting effects against some pomefruit diseases while no major negative side effect were reported (Costa *et al*., 2004; Rademacher, 2004).

The study on the mode of action pointed out that Prohexadione-Ca may interfere with dioxygenase catalyzing different specific 2-oxoglutarate dependent reactions, such GA20 oxidase and related enzyme that determine the reduction of shoot growth and with the flavanone 3-hydroxylase which determine an induction of resistance against pathogen infection. Prohexadione-Ca interferes also with the ascorbate-dependent ACC oxidase that determines a delayed senescence and reduced fruit.

The experiments carried out to evaluate the effect on vegetative and cropping performance and on fire blight suppression pointed out that the growth retardant

effect of Regalis is related to the cultivar, to the vigor of the considered combination and to the concentration used (Costa *et al*., 2004; Rademacher *et al*., 2004; Deckers *et al*., 2005; Maas, 2005) (Fig.4).

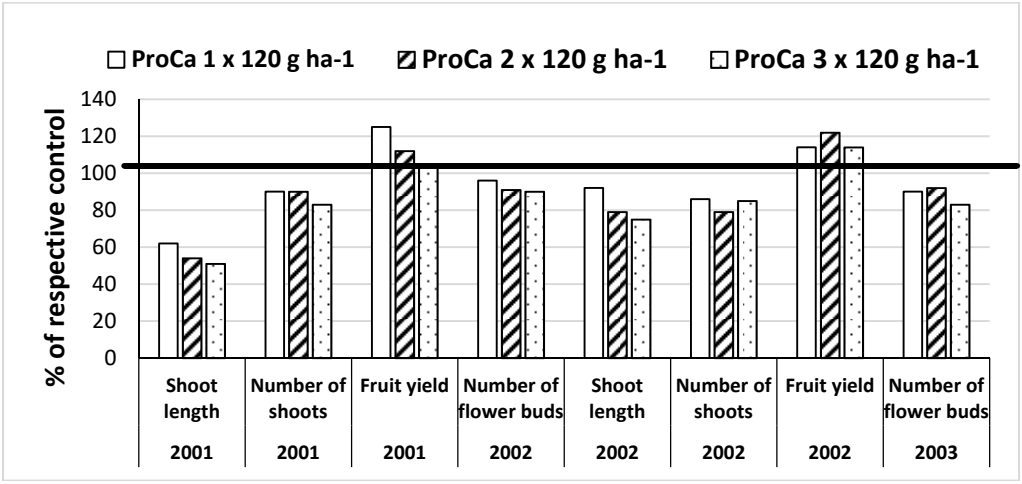

Figure 4. Effect of repeated application of Pro-Ca (1st application at the 3rd to 5th expanded leaf followed by 3 to 5 weeks sprays) on vegetative and reproductive parameters of cv Conference/Quince C tree (Source: Rademacher *et al*., 2004).

As far as the fire blight control, the application of Regalis showed an interesting activity and its effect was magnified, in some experimental conditions, when the Regalis was applied in combination with P10C (*Pantoea agglomerans)* (Vanneste *et al*., 2002; Costa *et al*., 2006; Vanneste, 2011) (Fig.5).

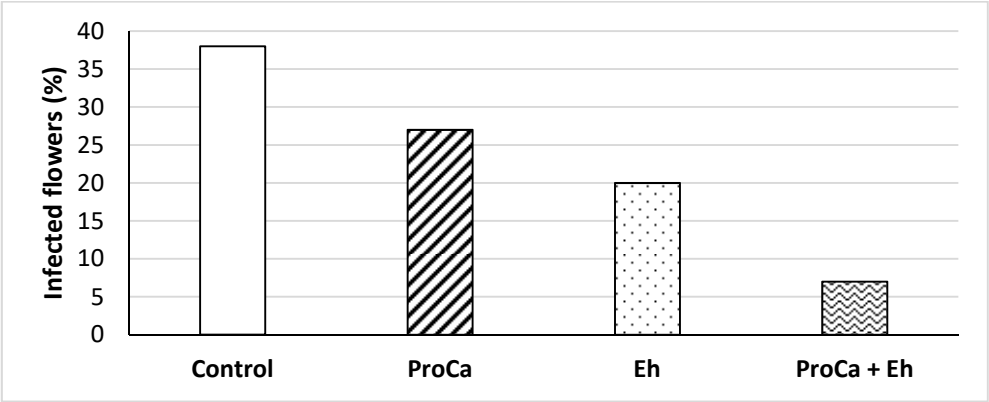

Figure 5. Effect of Pro-Ca and P10C (*Erwinia herbicola*) on Gala apple flower. (Source: Costa *et al*., 2002).

The negative effects that were detected, in some circumstances, on the return bloom represent the main concerns on the use of Regalis. The Regalis determine a reduction of the return bloom and this effect might depend upon the cultivar and the vigor of the considered combination.

Other PBRs like auxins and Ethephon, that might offer a certain growth control, are more effective in positively affect return bloom. NAA and/or Ethephon can be applied at 500-700 ml/ha in one or two application to control the vegetation and affect return bloom. NAA in a “paste” form can be also applied on the surface of important pruning cut to control apical dominance.

Fruit maturation, ripening and shelf-life management

Ethylene is considered the hormone that control the fruit ripening syndrome and PBRs able to control ethylene allow to control the fruit ripening in various pear cultivars.

In the recent years, several formulate able to interfere with ethylene biosynthesis or ethylene perception, have been found. Aminoethoxyvinilglicine (AVG; a.i. inReTain®) and 1-Methyl cyclopropene (1-MCP; a.i. in Smart FreshTM) are respectively inhibitors of enzyme important for ethylene biosynthesis or control the ethylene perception.

AVG initially experimentally tested to increase fruit set (Lombard and Richardson, 1982) find a commercial application in pre-harvest to control ripening. In fact, ReTain® can be used to extend the normal harvest period; it can be applied up to 4 weeks prior to harvest at 100-200 g/ha active ingredients in 1000 liters of spray volume to improve the fruit firmness and extends harvest period. ReTain® is also effective in reducing the pre-harvest abscission from 7 to 14 day as compared to untreated trees. As a result of extending the harvest period, fruit size may be increased. Pre-harvest applications of ReTain® also increased storability of pears and reduce senescence and storage disorders. The effectiveness is dependent upon application time, concentration and fruit ripening uniformity at the moment of application (Clayton *et al*., 2000; Dussi *et al*., 2002; Andreotti *et al*., 2004).

Smart FreshTM (a.i. 1 MCP) is instead a PBR that is applied in a gaseous form in post-harvest in Europe while in USA a pre-harvest formulate is also been tested (HarvistaTM 1.3 SC). The molecule has a higher affinity than ethylene for the ethylene receptor and is able to reduce the ethylene autocatalytic reaction determining a reduction of the ethylene amount available. Smart FreshTM showed to be very active in controlling ethylene production and fruit ripening (Tab. 1) (Ziosi *et al*., 2008).

Table 1. Influence of ripening stage and 1-MCP on ethylene production and fruit ripening. ‘Abbé Fétél’ pears were divided in in the classes 1 and 2 of homogeneous ripening (expressed as I_{AD}); 1-MCP was applied at 0.3 □l l^{-1} for 24 h (Source: Ziosi *et al.,* 2008).

Ripening stage		Ethylene (nl h^{-1} g^{-1} FW)				IAD		
		Months				Months		
		0	3	5	7	3	5	7
Class 1 (I_{AD} 2.3-2.0)	**C**	16.5	38.5	36.3		1.6	1.0	1.0
	T	9.1	24.4	33.6		1.8	1.5	1.5
Class 2 (I_{AD} 2.0-1.7)	**C**	22.6	19.2	34.2		1.1	0.7	0.6
	T	8.4	10.7	42.2		1.5	1.3	0.9

However, Smart Fresh™, from one side indicated the capability to slow down the fruit softening rate and a reduction of fruit losses during storage, although underlined some concerns about the capability to recover the ripening capacity of the treated pear fruit after cold storage (Rizzolo *et al*., 2014). In fact, in some trials, 1-MCP treated fruits did not reach the typical skin color and the firmness was maintained at very high level (Vidoni *et al*., 2013; 2015; Wang *et al*., 2015). In addition fruits harvested at different ripening stage showed that during storage the fruit-ripening trend is different between the two fruit ripening classes and the reached values remain always lower for 1-MCP treated fruits (Fig.7). Some researchers tested the possibility to prevent ripening blockage in 1-MCP treated ‘Abbé Fetel’ pears by temperature management (Cucchi and Regiroli, 2011; Folchi *et al*., 2014; Wang *et al.* 2015).

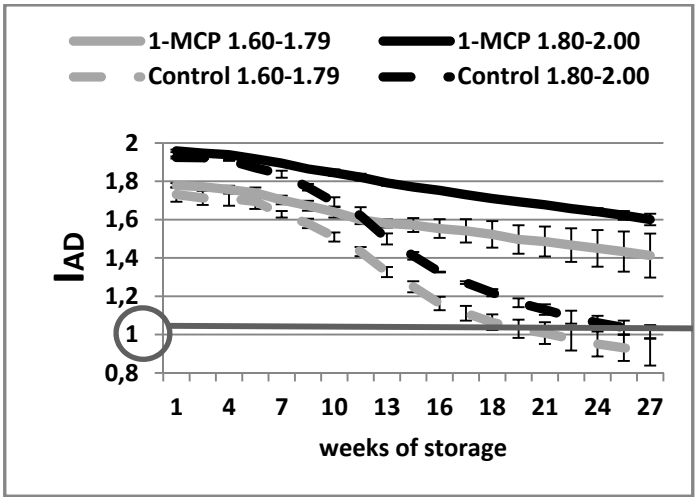

Figure 7. Differences between fruits harvested at a different ripening stage (expressed as Index of Absorbance difference (IAD) determined with the DA-Meter). Values for the riper fruits ranged from IAD **1.6 to 1.79** and for the less ripe from IAD **1.80 to 2.00.** Control and 1-MCP treated fruits maintain during the storage period the ripening value differences they had at harvest and fruits 1-MCP treated have a completely different trend as compared to the control fruits harvested at the same ripening stage.

Nowadays, NAA, AVG, and 1-MCP (HarvistaTM 1.3 SC) are efficient tools for controlling pre-harvest fruit drop, extending harvest window, and increasing storability in pears. Application timing, rate, and fruit harvest maturity are critical for efficacy and reducing the possible negative effects of the PBRs on pears.

It has to be pointed out that also auxins might play an important role in the maturation and ripening process of pear fruit as well as the growth substances polyamines that have been tested successfully in other fruit species (Bregoli *et al*., 2005; 2006). There is a close relationship between auxin, polyamines and ethylene. In pear, for example, NAA has been thought to stimulate the production of ethylene and can hasten fruit maturation and softening. It is known in the practice that the use of auxin-like formulates, such as AF96 induced positive effect on the post-harvest management.

Final remarks

Plant bioregulators (PBRs) that mimic and function as the naturally occurring plant hormones affecting vegetative and fruiting parameters, improving yield, quality and postharvest life and representing an important tool in fruit tree management.

In pear, the use of PBRs started several years ago and nowadays is still an important and necessary cultural management operation. Auxin-like formulates were found useful in increasing fruit set, overcoming biennial bearing and counteracting pre-harvest drop. Gibberellins and cytokinins also increased fruit yield and ameliorate shape and quality of fruit. Other areas of fruit production where PBRs are especially useful include prevention of pre-harvest fruit drop, control of vegetative growth, enhancement of flower bud formation and control of fruit ripening.

In fact, in recent years, the introduction of the Prohexadione-calcium, an inhibitor of gibberellin biosynthesis, allow to control shoot growth and altering plant metabolism to impart resistance to insects and diseases. More recently, other compounds as aminoethoxyvinylglycine, an ethylene biosynthesis inhibitor and 1-methylcyclopropene (1-MCP), a competitive inhibitor of ethylene, allow extending harvest window, postharvest life improving storage quality of fruit.

The research on the use of PBRs in pear is still ongoing. Among the newly proposed PBRs, the naturally occurring hormone, Abscisic acid can potentially overcome plant stress, enhancing fruit color, and early fruit drop, but its effectiveness in pears needs to be confirmed with further research.

However, it has to be considered that the use of PBRs is a difficult technique. In fact, plant bioregulators have the peculiarity that, in some circumstances, the same active ingredient can induce different responses depending on the application time and rate used. It has to be carefully considered that the PBRs effectiveness is affected by several internal and external factors like cultivar, tree vigor and yield, climatic conditions during the application period as well as the application methodology used. Of course this complicates the scenario and PBRs must be used in a proper way to obtain the desired results.

As a conclusive remark, the plant growth or bioregulators (PGRs or PBRs) in pear are an important tool, although, they must be considered a part of a larger portfolio of options to be integrated into a whole sustainable, systematic approach program for controlling vigor and improving cropping. In anycase a proper use of the PBRs require a deep understanding of the physiological mechanism of the process to control and the nature of the active ingredient of the used principle to obtain the main desired effect and avoid the collateral negative side-effects.

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