

Controlling Peach Fruit Quality by Interfering with Ethylene Production with Polyamines and Jasmonates

Patrizia Torrigiani* • Vanina Ziosi • Anna Maria Bregoli • Guglielmo Costa

Dipartimento di Colture Arboree, Università di Bologna, Via Fanin 46, 40127 Bologna, Italy

Corresponding author: * patrizia.torrigiani@unibo.it

ABSTRACT

In climacteric fruit, the control of ripening mainly relies on ethylene production and/or action, although there are some parameters that do not seem to strictly respond to this hormone. The peach fruit is well characterized from the physiological and molecular point of view and thus it offers a good fruit model system to study the relationship between ripening and quality. Here we review the results of the application of two kinds of natural growth regulators, polyamines (PAs) and jasmonates (JAs). Although characterized by substantially different biological activities, both are able to interfere with ethylene synthesis and perception, and, as a consequence, to alter the progression of ripening and the evolution of the main fruit quality traits. On the basis of endogenous levels of PAs and JAs in the fruit, experimental protocols aimed at manipulating these levels were established. This article reports the main results obtained in relation to fruit ripening and quality, and functional properties. Field application to peach fruit in late developmental stage (S3-S4) of the PAs putrescine (Pu), spermidine (Sd) or spermine (Sm) leads almost invariably to ripening delay and prolonged shelf-life. This is due to inhibition of ethylene biosynthesis/production, inhibition of cell wall-dismantling activity/retention of flesh firmness (FF), reduced soluble solids concentration (SSC). Equally, in the case of JAs, their application *in planta* causes a ripening delay, as shown by reduced ethylene biosynthesis/production and firmness retention probably due to a trade-off between growth and defence. Although likely via different signal transduction pathways, PAs and JAs, by interfering with ethylene biosynthesis and perception, lead to a less ripe fruit and, consequently, they are very promising compounds for quality control in the postharvest handling chain. For practical purposes, besides optimizing application strategies, commercial products containing PAs would be highly desirable.

Keywords: flesh firmness, ethylene, fruit quality and ripening, field treatments, jasmonates, peach, polyamines, nectarine

Abbreviations: ACO, 1-aminocyclopropane-1-carboxylic acid oxidase; ACS, 1-aminocyclopropane-1-carboxylic acid synthase; AVG, aminoethoxyvinylglycine; FF, flesh firmness; JA, jasmonate; MJ, methyl jasmonate; PA, polyamine; PDJ, propyl dihydrojasmonate; Pu, putrescine; Sd, spermidine; Sm, spermine; SSC, soluble solids concentration; TA, titratable acidity

CONTENTS

INTRODUCTION.....	85
MODULATING PEACH FRUIT RIPENING/QUALITY BY POLYAMINES	85
Polyamines are essential for plant growth and survival	85
Field polyamine application results in firmness retention	86
Postharvest polyamine treatments differentially affect ripening	87
EXOGENOUS JASMONATES IMPACT PEACH FRUIT RIPENING	88
Jasmonates are needed for plant development and immunity	88
Field treatments: a possible trade-off between growth and defence	88
CONCLUSIONS.....	89
ACKNOWLEDGEMENTS	89
REFERENCES.....	89

INTRODUCTION

The concept of sustainable agriculture passes also through the availability and use of naturally occurring molecules capable of controlling physiological processes. For instance, compounds able to modulate fruit quality and of being integrated into cell metabolism without leaving noxious residues in the produce or in the soil are welcome in horticultural crop practices. The aliphatic polyamines (PAs) and the class of molecules known as jasmonates (JAs) are plant growth regulators that appear to fulfil these requirements. Since in both cases their use in the field has not been authorized, further studies are needed to definitely demonstrate their efficacy when used in postharvest and under field conditions, and their safety. Here we review the results of several years of experimental work on peach and nectarine

regarding the effects, as explored at agronomical, physiological and molecular levels, of PAs and JAs on fruit development and ripening. The model system we adopted is the peach drupe since many physiological, biochemical and molecular parameters are now well characterized (Bonghi and Trainotti 2006) and the knowledge of these parameters is fundamental in order to apply effective pre- and post-harvest ameliorative strategies.

MODULATING PEACH FRUIT RIPENING/QUALITY BY POLYAMINES

Polyamines are essential for plant growth and survival

PAs constitute a class of ubiquitous plant growth regulators,

characterized by their polycationic nature and their ability to form electrostatic bindings with biological macromolecules aimed at stabilizing them and favouring their function (Cohen 1998); in fact, PAs are known to play a positive role in plant growth and differentiation (Bagni and Torrigiani 1992) and in stress/defence responses (Alcazar *et al.* 2006; Pang *et al.* 2007; Groppa and Benavides 2008; Kusano *et al.* 2008). The diamine precursor of the higher PAs spermidine (Sd) and spermine (Sm) is putrescine (Pu); the latter is synthesized indirectly from arginine via arginine decarboxylase (ADC) and/or directly from ornithine via ornithine decarboxylase (ODC). S-adenosylmethionine (SAM), which is also precursor for ethylene biosynthesis, is decarboxylated by SAM decarboxylase (SAMDC); decarboxylated SAM furnishes the aminopropyl groups needed for Sd and Sm biosynthesis from Pu and Sd via Sd (SPDS) and Sm synthase (SPMS), respectively. Besides their biosynthetic enzymes, cellular PA levels are also regulated by the action of di- and polyamine oxidases (Cona *et al.* 2006) and by conjugation to hydroxycinnamic acids (Martin-Tanguy 1985).

PA levels change whenever cells change their physiological status upon perception of an endogenous (hormonal) or exogenous (environmental) stimulus. Indeed, during fruit development and ripening, endogenous PA concentrations undergo dramatic changes, the maximum levels occurring during the early growth stages (cell division phase) and the minimum levels at ripening (Liu *et al.* 2006; Torrigiani *et al.* 2008); in climacteric fruit such as peach, pear and others, minimum PA levels coincide with climacteric ethylene production.

The physiological features of PAs as growth regulators render them suitable for studies in horticulture, as first demonstrated in apple by the pioneering work of Costa and Bagni (1983). The latter authors showed that field PA applications at flowering resulted in substantial increases in fruit set and final yield at harvest. Afterwards, many other fruit species have been treated with PAs, mainly in postharvest, showing, almost invariably, that these compounds exert a beneficial effect on postharvest decay and on fruit shelf-life (Valero *et al.* 2002; Liu *et al.* 2006; Torrigiani *et al.* 2008). Thus, these molecules apparently have great potential though much remains to be investigated, especially as regards field trials.

Field polyamine application results in firmness retention

The first approach to the investigation of the potential of PAs in the control of fruit ripening in peach was a field trial (Bregoli *et al.* 2002). Pu, Sd and Sm at different concentrations were applied by spraying to peach trees (Redhaven) at mid-S3 (pre-harvest) in order to check *in planta*, under field conditions, what the impact of these molecules on ethylene production was, and what effects these growth regulators exerted on peach fruit ripening and quality. Later, and on the basis of the results obtained in peach, the study was extended to nectarines (Stark Red Gold) and included other fruit developmental stages, each corresponding to different endogenous PA concentrations in the mesocarp (Kushad 1998; Ziosi *et al.* 2003; Ziosi *et al.* 2006a). Thus, PA treatments at S1, mid-S3 and late-S3 stages were performed showing that early and late applications all lead to a delay in ripening.

In Redhaven peaches, PA application prior to the start of ripening (19 d before harvest, mid-S3) led to strong inhibition of ethylene biosynthesis and retention of FF (Table 1; Bregoli *et al.* 2002). A clear PA inhibitory effect on ethylene emission was shown in peach fruit after a whole canopy/branch field treatment; at that time, similar results had been only reported in Japanese apricot "painted" with exogenous PAs 20 days before harvest (Paksasorn *et al.* 1995). In peach, all PA concentrations strongly reduced or even nullified ethylene production, and the extent of ethylene inhibition seemed to depend upon the type and concentration of the PA applied: Sd was more efficient than Pu and Sm. PA-

treated fruit also showed a lower SSC.

In Stark Red Gold nectarine, field application (mid-S3, 28 d before harvest) of both Pu and Sd strongly reduced ethylene production, though generally less than in the previous case, confirming previous observations with Redhaven peaches (Table 2; Torrigiani *et al.* 2004); however, in this case, effects appeared independent of PA concentration or type. Since PAs reduced ethylene production, consequent abscission control was expected. In fact, while a transient reduction in fruit abscission was registered in Pu-treated fruit, the highest Sd concentration exerted a lasting abscission control. The different biological activity of the two PAs could account for these differences: only the PA with the higher biological activity, i.e., the triamine Sd (Hanfrey *et al.* 2002), maintains abscission control. In all PA-treated nectarines, SSC was lower and flesh softening was delayed (Table 2) to a greater extent than in Redhaven peaches. This last aspect was confirmed in a further work on nectarine where, besides inhibition of ethylene production and fruit drop (Sd), FF was retained (3-4 times relative to controls) following late-S3 (15 d before harvest) Pu and Sd field treatment (Table 3; Ziosi *et al.* 2006a). For both mid- and late-S3 treatments, data on ethylene production in nectarine were corroborated by gene expression analysis; results showed that the expression of ethylene biosynthetic genes *PpACS* and/or *PpACO1* was down-regulated at harvest in PA-treated mesocarp; moreover, PAs also affected the expression of ethylene receptors genes *PpETR1* and *PpERS1*.

It is worth noting that, in nectarines, earlier PA application, at the same concentrations, delayed ethylene production more markedly than the later ones (Tables 2 and 3; Torrigiani *et al.* 2004; Ziosi *et al.* 2006a). This was attributed to the fruit developmental stage at the time of treatment. Indeed, in nectarine, PA treatment 7 d before harvest (S4) did not result in any changes in ethylene production or

Table 1 Ethylene production (nl h⁻¹ g⁻¹ FW), flesh firmness (FF, N), soluble solids concentration (SSC, °Brix) and Pu, Sd and Sm concentration (nmol g⁻¹ FW), expressed as percentage compared with controls (100%), in Redhaven peaches at harvest. Fruit were treated at mid-S3 stage (19 d before harvest) with Pu, Sd or Sm (modified from Bregoli *et al.* 2002).

	Ethylene	FF	SSC	Pu	Sd	Sm
10 mM Pu	19	164	100	58	65	59
0.1 mM Sd	7	171	93	92	100	100
1 mM Sd	0	164	93	50	49	59
5 mM Sd	3	164	96	58	57	59
2 mM Sm	38	179	100	63	78	88

Table 2 Ethylene production (nl h⁻¹ g⁻¹ FW), flesh firmness (FF, N), soluble solids concentration (SSC, °Brix), titratable acidity (TA), fruit abscission and PA concentration (nmol g⁻¹ FW), expressed as percentage compared with controls (100%), in Stark Red Gold nectarines at harvest. Fruit were treated at mid-S3 stage (28 d before harvest) with Pu or Sd (modified from Torrigiani *et al.* 2004).

	Ethylene	FF	SSC	TA	Abscission	PAs
5 mM Pu	32	300	89	110	100	100
10 mM Pu	13	400	86	121	100	100
20 mM Pu	34	380	83	109	100	100
0.5 mM Sd	26	500	89	100	40	100
1 mM Sd	16	460	91	100	90	100
2 mM Sd	23	200	90	100	40	100

Table 3 Ethylene production (nl h⁻¹ g⁻¹ FW), flesh firmness (FF, N), soluble solids concentration (SSC, °Brix), fruit abscission and Pu concentration (nmol g⁻¹ FW), expressed as percentage compared with controls (100%), in Stark Red Gold nectarines at harvest. Fruit were treated at late-S3 stage (15 d before harvest) with Pu, Sd or AVG (modified from Ziosi *et al.* 2006a).

	Ethylene	FF	SSC	Abscission	Pu
10 mM Pu	82	428	100	100	200
0.1 mM Sd	70	314	100	78	200
0.32 mM AVG	64	257	100	52	100

fruit quality (unpublished) suggesting that, *in planta*, the later the PA application, the weaker the effect on ripening. This may be the result of the fact that, when the molecular processes which underlie ripening are too far advanced, they cannot be effectively counteracted.

Surprisingly, no dose-dependent responses were ever observed in PA-treated fruit. This was explained as possibly due to saturation of PA-binding sites at the cell wall (galacturonic units of pectins; D'Orazi and Bagni 1987) and/or membrane levels (Tassoni *et al.* 1998) in the epicarp that constitutes the barrier which may control and limit the entrance of PAs in the tissue. Nevertheless, even at tissue concentrations much lower than those administered (see below), PAs were able to exert a dramatic effect on ethylene production by modifying the expression level of the ethylene biosynthetic and perception genes. It has been proposed that ethylene and PA biosynthetic pathways may compete for their common precursor SAM as shown in *Prunus avium* shoot cultures (Biondi *et al.* 1990). Actually, SAM concentration well exceeds that needed for PAs and ethylene synthesis (Ravanel *et al.* 1998) so that it is unlikely that such competition does occur.

The possibility that PAs act directly upon the fruit quality parameters examined (softening, sugar content, etc.) cannot be excluded. As far as FF is concerned, in Golden Delicious and McIntosh detached apples, it has been proposed that exogenous PAs affected fruit softening through rigidification of cell walls by inhibiting polygalacturonases rather than through interaction with ethylene synthesis (Kramer *et al.* 1991) while in plum an interference with cell dismantling enzyme activities was found (Kahn *et al.* 2007). Pu infiltrated in ripe peaches increased FF thereby delaying the ripening process (Martinez-Romero *et al.* 2000). Equally, in damaged plum fruits, application of Pu increased firmness, accompanied by an increase in the acid-insoluble conjugated PAs; the authors suggested that their binding to cell walls may be responsible for the lesser fruit flesh deformation (Perez-Vicente *et al.* 2002). However, while in Redhaven peach insoluble conjugated PAs occur (Bregoli *et al.* 2002), in nectarine, they were not detected during fruit ripening (Torrighiani *et al.* 2004; Ziosi *et al.* 2006a). Thus, at least in nectarines, the control of firmness seems to be unrelated to the cross-linkages between PA conjugates and cell walls constituents. On the contrary, it is likely that the control of FF exerted by PAs is mediated by ethylene, which plays a predominant role in this phenomenon by acting on PG activity and gene expression (Bonghi and Trainotti 2006). In Redhaven peaches, PG is one of the genes up-regulated at the transition between the pre-climacteric to the climacteric stage (Trainotti *et al.* 2006).

The relationship between PAs and fruit quality has been approached by genetic engineering in tomato giving rise to interesting results. Transgenic tomatoes overexpressing a yeast SAMDC gene and over-accumulating Sd and Sm have been shown to accumulate more lycopene and to have prolonged vine life (Metha *et al.* 2002). Transcript profiling has shown changes in gene expression consistent with the role of PAs as stress protecting and antisenesescence effectors (Srivastava *et al.* 2007); metabolite NMR analysis indicates changes in primary metabolite pathways that result in better juice and nutritional properties (Mattoo *et al.* 2006). Interestingly, engineered accumulation of higher PAs in tomato restored metabolic activity even at late stages of fruit ripening, reviving cellular programs underlying N:C signalling, energy and glucose metabolism (Mattoo and Handa 2008). Although genetic modification in fruit tree species remains a difficult tool, these results encourage further studies on PA application in horticulture.

Rather surprisingly, the impact of exogenous PAs on the endogenous PA pool in ripe Redhaven peaches following PA treatment, was inhibitory, so that Pu, Sd and Sm concentrations were lower (10-50%) compared with untreated controls (Table 1; Bregoli *et al.* 2002). In nectarine, the mid-S3 treatment led to no change in PA concentration in the ripe fruit (Torrighiani *et al.* 2004), while the late-S3 treatment led

to a doubling of the PA concentration at harvest (Ziosi *et al.* 2006a). In any case, the observed changes are modest considering the relatively high concentrations of exogenous PAs (up to 20 mM) compared with the endogenous ones (60-80 μ M total PAs). Nevertheless, even with no or modest changes in flesh PA concentration at ripening, the amines exerted their re-juvenating effect and in fact the ripening process was profoundly affected. Their scarce influence on endogenous levels suggests that, despite the enormous concentration gradient, most of the applied amines were retained in the epicarp, where they probably underwent degradation by environmental factors. Actually, the fact that the endogenous PA concentration was scarcely affected is a positive aspect, especially considering that high levels of PAs in the fruit mesocarp could be an undesirable side-effect for consumption under particular regimes (Bardóc 1995).

From these data, the notion that these naturally occurring substances can be a useful tool for controlling fruit ripening in the field arises. No dose-dependent responses were in general observed, suggesting that the lesser PA concentrations (i.e., 5 mM Pu and 0.1 mM Sd) may be suitable. These studies also provide new information on the molecular basis of PA action, since, for the first time in a climacteric fruit, it was demonstrated that PAs affect the expression of ethylene biosynthetic and perception genes (Ziosi *et al.* 2009).

Postharvest polyamine treatments differentially affect ripening

A more frequent experimental approach to study the potential of PAs for modulating fruit quality is their post-harvest application. In a comparative study, detached Redhaven peach and Stark Red Gold nectarines at two ripening stages (commercial harvest and tree-ripe) were utilized for studying the relationship between PAs and ethylene, and effects on fruit quality (Bregoli *et al.* 2006). Preliminarily, labelled Pu was used as a marker to check the extent of diamine uptake by the fruit. Surprisingly, the timing of Pu uptake differed in the two cvs: in nectarine, labelled Pu was promptly and increasingly taken up in the epicarp and, to a small extent, translocated into the mesocarp. In peach, the amount of label initially recovered in the epicarp was several-fold lower than in nectarine but, later, was two-to-three-fold higher in the mesocarp; beyond the first 30 min of incubation, the amount of label recovered became similar in the two cvs. This behaviour was assumed to depend upon the different histological features of the epicarp of peach and nectarine (King *et al.* 1987; Masia *et al.* 1992).

In detached nectarine, dipped for 1 min in PA solutions, Pu inhibition of ethylene production was transient in firmer fruit, while it increased with time in the softer ones, suggesting that firmer fruit are less permeable or sensitive to Pu. Moreover, since the extent of ethylene production was more than twice in softer than in firmer nectarines, the inhibitory effect of Pu in relative terms appeared far stronger in the former than in the latter. At both ripening stages, the negative effect of Sd on ethylene production in nectarine increased with time in accord with the notion that this triamine, though supplied 10-fold less concentrated than the diamine Pu, and despite the fact that its three positive charges probably impaired its uptake and translocation, has a stronger biological activity than Pu. The increase in transcript amount of *PpACO1*, which is a marker of ripening, was delayed by both PAs, by 2 days in the mesocarp of softer nectarines, in accord with the inhibition of ethylene production.

In contrast with the above results, in detached Redhaven peaches, exogenous PAs did not substantially affect ethylene production and fruit quality. The higher efficacy of PAs in nectarine than in peach was imputed to the fact that Pu is taken up to a higher extent in the former than in the latter (Bregoli *et al.* 2006). The scarce effect of PAs on firmness and SSC in both cultivars, especially in softer fruit, was ex-

plained on the basis of the fact that treatments were carried out on fruit in late ripening stages (commercial harvest and tree-ripe), such that cell wall degradation processes and sucrose accumulation were probably too far advanced to be contrasted. Both cultivars, at both the ripening stages, responded in the expected manner (i.e., with inhibition of ethylene production) to the ACS activity specific inhibitor AVG (Yu and Yang 1979), confirming that the detached fruit model was working properly and that peach, under these experimental conditions, is less sensitive than nectarine to exogenous PAs. Nevertheless, postharvest PA application in several other fruit species has been generally successful, especially when PAs were pressure-infiltrated (Martinez-Romero *et al.* 2000; Perez-Vicente *et al.* 2002; Petkou *et al.* 2004; Liu *et al.* 2006; Torrigiani *et al.* 2008).

The detached fruit model was compared with a fruit explant model. The latter has been used, though less frequently, for ripening physiology studies since experimental conditions may be strictly controlled. Results show that only Sd, but not Pu, was able to inhibit ethylene biosynthesis and sucrose accumulation in ripe peach and nectarine explants (Bregoli *et al.* 2006). Also in this case, proof of the fact that the explant model was working well came from the strong inhibitory effect of AVG on ethylene biosynthesis. Results obtained with explants only partially overlap with those from intact fruit, likely due to the interfering wound response of explants, and suggest that *in vitro* models have to be evaluated with caution before drawing general conclusions.

In conclusion, detached nectarine, especially at the riper stage, respond better than Redhaven peach, which is essentially insensitive, to Pu and Sd treatment (dipping) in terms of ethylene production; in all cases fruit quality was only modestly affected. Nevertheless, though depending upon fruit species, cultivar, physiological stage and mode of application, aliphatic PAs may exert a profound effect on fruit ripening also in postharvest.

EXOGENOUS JASMONATES IMPACT PEACH FRUIT RIPENING

Jasmonates are needed for plant development and immunity

Jasmonic acid and its derivative methyl jasmonate (MJ), the best known JAs, are ubiquitous plant hormones which mediate many plant responses to biotic and abiotic stresses (Wasternack 2007). They are synthesized via multiple steps in the octadecanoid pathway starting from α -linolenic acid and with allene oxide synthase (AOS) as the first specific key enzyme. JA treatment leads to up-regulation of JA-responsive genes (JRGs) and to the synthesis of JA-induced proteins (JIPs), such as enzymes involved in JA biosynthesis and in secondary metabolism, as well as in stress-protective and cell wall-related proteins (Creelman and Mullet 1997).

JAs are also involved in developmental processes, since in *Arabidopsis*, JA-deficient biosynthetic mutants exhibit abnormal male reproductive organ formation (Wasternack 2007): Moreover, JA levels are developmentally regulated during fruit growth and ripening (Fan *et al.* 1998a; Kondo *et al.* 2000; Ziosi *et al.* 2008). The growing importance of JAs and their impact on horticultural crop protection from

environmental stress, postharvest decay and handling has been recently reviewed (Rohwer and Erwin 2008). In fruit, JAs assume particular relevance since they induce, when exogenously supplied, secondary metabolite biosynthesis as a stress response, and this often translates in an improvement of fruit quality and nutritional properties. For instance, JAs are able to stimulate β -carotene and anthocyanin biosynthesis, and volatile emission in apple (Pérez *et al.* 1993; Kondo *et al.* 2005; Rudell and Mattheis 2008), flavonoid accumulation and antioxidant activity in blackberry and raspberries (Chanjirakul *et al.* 2006; Wang *et al.* 2008), and resveratrol biosynthesis in grape (Vezzulli *et al.* 2007). Moreover, MJ reduces chilling injury symptoms in mangoes (González-Aguilar *et al.* 2001) and inhibits *Colletotrichum acutatum* infection in loquat (Cao *et al.* 2008). Nevertheless, reciprocal relationships between JAs and ethylene are not well established. In particular, during fruit ripening, contradictory results have been reported, and the effects of JAs on ethylene seem to strongly depend, besides fruit species and cultivar, upon physiological stage of application (Fan *et al.* 1998b; Khan and Singh 2007; Kondo *et al.* 2007; Ziosi *et al.* 2008). Here, results of field treatments of nectarines performed with the aim to evaluate JA effects on fruit ripening and quality are reviewed.

Field treatments: a possible trade-off between growth and defence

Pre-harvest applications under field conditions of MJ (100-200 ppm = 0.44-0.88 mM) and its synthetic analogue PDJ (50 ppm = 0.20 mM) to Stark Red Gold nectarine trees bearing fruit at different developmental stages (S1, S1/S2, S3 and S4) impact ripening, as deduced by ethylene production, changes in FF, SSC, flesh colour and PA concentration, leading to modulation of fruit quality. Changes in these parameters are supported by variations in correlated gene expression. Fruit growth monitoring in JA-treated fruit did not reveal any change in fresh weight or diameter, but, in all cases, treated fruit at harvest, produced less ethylene, retained FF, and in most cases, exhibited lower SSC compared with controls (Ziosi *et al.* 2006b; 2008; unpublished results). At harvest, S1-treated fruit produced 33% ethylene relative to controls assumed as 100% and were firmer (FF 46 vs 35 N); very similar results were obtained with S1/S2-treated fruit. In the case of S3 and S3/S4 treatments, **Table 4** shows that, at harvest, ethylene production was dramatically inhibited in the mesocarp of treated fruit, together with *PpACO1* transcript accumulation; FF was retained up to 3-fold and SSC was lower than in controls. On the other hand, in the same fruit, *PpAOS1* mRNA and the concentration of jasmonic acid were up-regulated according to the notion that fruit ripening was delayed (Ziosi *et al.* 2008). The latest JA application (S4 stage) was almost ineffective, leading to the conclusion that the more advanced the ripening process the less active the compound. Opposite effects were observed in Redhaven peach trees sprayed repeatedly with 10 mM MJ (one hundred-fold the concentration used in the experiments described above): fruit colour was enhanced and firmness was reduced (Janoudi and Flore 2003) thus confirming the importance of the concentration applied.

A transcriptome analysis conducted on ripe fruit treated with MJ at the S3 stage (21 d before harvest), showed that indeed, in the long-term, MJ down-regulated ripening-rela-

Table 4 Ethylene production ($\text{nl h}^{-1} \text{g}^{-1} \text{FW}$), flesh firmness (FF, N), soluble solids concentration (SSC, °Brix), Pu, Sd ($\text{nmol g}^{-1} \text{FW}$) and jasmonic acid (JA, $\text{ng g}^{-1} \text{DW}$) concentration, expressed as percentage compared with untreated controls (100%), in JA-treated Stark Red Gold nectarines; *PpACO1* and *PpAOS* transcript up-regulation and down-regulation relative to controls are indicated by (+) and (-), respectively. Fruit were treated with 100 ppm MJ and 50 ppm PDJ at mid-S3 and late-S3 stages (21 and 14 d before harvest, respectively; Ziosi *et al.* 2008); nd, not determined.

		Ethylene	FF	SSC	Pu	Sd	JA	<i>PpACO1</i>	<i>PpAOS</i>
S3	MJ	6	285	84	116	100	130	(-)	(+)
	PDJ	10	279	87	132	125	130	(-)	(+)
S3/S4	MJ	40	186	67	nd	nd	nd	nd	nd
	PDJ	29	179	87	nd	nd	nd	nd	nd

ted genes such as *PpACO1* and polygalacturonase (Bonghi and Trainotti 2006), and up-regulated a number of stress-related genes, such as AOS itself, and cell wall metabolism-related genes (Ziosi *et al.* 2008) confirming indeed the agronomical and biochemical observations. PA biosynthetic genes are among the genes up-regulated by JAs (Biondi *et al.* 2001). In fact, PA levels were enhanced in the epicarp and mesocarp of JA-treated fruit (Ziosi *et al.* 2009) lending support to the notion that JA-treated fruit were less ripe than controls. This means that the physiological effect induced by MJ in nectarine was profound, as deduced by its consequences at the gene expression level. In particular, up-regulation of several defence genes together with ripening delay, indicates that likely resources were diverted from developmental to defence programme consistent with a trade-off between the two (Schmidt and Baldwin 2006).

CONCLUSIONS

Exogenous PA application to developing peach fruit dramatically inhibits ethylene production, leads to a ripening delay, and interferes with fruit quality. On the other hand, inhibition of ethylene biosynthesis and perception results in alterations of PA biosynthetic gene expression (Bregoli *et al.* 2005) confirming the notion that a strict cross-talk between the two growth regulators occurs during ripening. Directly or indirectly, fruit quality traits, such as FF, SSC and titratable acidity, are also positively influenced by PAs. In pomegranate it has been shown that among the metabolites influenced by PAs, there are different classes of phenolic compounds, carotenoids, ascorbic acid and fatty acids (Mirdeghan *et al.* 2007). All are related to fruit nutritional properties, which are acquiring increasing relevance for consumers. In transgenic tomato over-accumulating Sd and Sm, besides fruit quality-related metabolism, anabolic pathways are positively affected, in particular the N/C sensing. This is in agreement with the antisenescence properties of PAs, and compatible with a high fruit metabolic status. Information arising from field PA applications, at early phenological stages, may open new perspectives for the use of these compounds in the control of fruit development (fruit set, retention, yield, size) and ripening, and in counteracting biotic and abiotic stresses as revealed by their effects on cell wall-related enzyme activities and phenolic contents. Moreover, PAs, being biological components, represent an environmentally compatible tool as they can be metabolized by fruit cells. Although endogenous PA concentrations increase in treated fruit, they remain well below toxic levels. In addition, more extensive metabolic profiling is needed in order to gain deeper insight into the nutritional attributes of PA-enriched/treated fruit. Although this review provides strong evidence for the efficacy of PAs as a tool for ameliorating the whole-fruit productive chain, PA-based commercial products are still lacking. Thus rendering this aspect of PA research difficult and expensive. Equally, the use of JA appears to be a promising tool for field application aimed at the control of pre-harvest fruit ripening and quality. Results described here show that JAs can successfully be used *in planta* to control ripening and defence, and this may have positive consequences in postharvest. Present information arises from JA application in a field environment, where plants have to cope with multiple biotic and abiotic elicitors, but nevertheless opens new perspectives for the use of JAs in the control of fruit ripening and defence.

ACKNOWLEDGEMENTS

Research on PA and JA involvement in peach development and ripening was supported by funds from the Italian Ministry of University and Scientific Research to P.T. and G.C. (PRIN 1998, 2000, 2002, 2003 and 2005). The authors wish to thank Prof. S. Biondi for helpful discussions.

REFERENCES

- Alcázar R, Marco F, Cuevas JC, Macarena P, Ferrando A, Carrasco, Tiburcio AF, Altabella T (2006) Involvement of polyamines in plant response to abiotic stress. *Biotechnological Letters* **28**, 1867-1876
- Bagni N, Torrigiani P (1992) Polyamines: a new class of growth substances. In: Karssen CM, Van Loon LC, Vreugdenhil D (Eds) *Progress in Plant Growth Regulation*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 264-275
- Bardóc S (1995) Polyamines in food and their consequences for food quality and human health. *Trends in Food Science and Technology* **6**, 341-346
- Biondi S, Diaz T, Iglesias I, Gamberini G, Bagni N (1990) Polyamines and ethylene in relation to adventitious root formation in *Prunus avium* shoot cultures. *Physiologia Plantarum* **78**, 475-483
- Biondi S, Scaramagli S, Capitani F, Altamura MM, Torrigiani P (2001) Methyl jasmonate upregulates biosynthetic gene expression, oxidation and conjugation of polyamines, and inhibits shoot formation in tobacco thin layers. *Journal of Experimental Botany* **52**, 231-242
- Bonghi C, Trainotti L (2006) Genomic tools for a better understanding of the fruit ripening process. *Steward Postharvest Review* **2**, 1-10
- Bregoli AM, Scaramagli S, Costa G, Sabatini E, Ziosi V, Biondi S, Torrigiani P (2002) Peach (*Prunus persica* L.) fruit ripening: aminoethoxyvinylglycine (AVG) and exogenous polyamines affect ethylene emission and flesh firmness. *Physiologia Plantarum* **114**, 472-481
- Bregoli AM, Ziosi V, Biondi S, Rasori A, Ciccioni M, Costa G, Torrigiani P (2005) Postharvest 1-methylcyclopropene application in ripening control of 'Stark Red Gold' nectarines: temperature-dependent effects on ethylene production and biosynthetic gene expression, fruit quality, and polyamine levels. *Postharvest Biology and Technology* **37**, 111-121
- Bregoli AM, Ziosi V, Biondi S, Bonghi C, Guglielmo C, Torrigiani P (2006) A comparison between intact fruit and fruit explants to study the effect of polyamines and aminoethoxyvinylglycine (AVG) on fruit ripening in peach and nectarine (*Prunus persica* L. Batch). *Postharvest Biology and Technology* **42**, 31-40
- Cao S, Zheng Y, Yang Z, Tang S, Jin P, Wang K, Wang X (2008) Effect of methyl jasmonate on the inhibition of *Colletotrichum acutatum* infection in loquat fruit and the possible mechanisms. *Postharvest Biology and Technology* **49**, 301-307
- Chanjirakul K, Wang SY, Wang CY, Siriphanich J (2006) Effect of natural volatile compounds on antioxidant capacity and antioxidant enzymes in raspberries. *Postharvest Biology and Technology* **40**, 106-115
- Cohen SS (1998) *A Guide to the Polyamines*, Oxford University Press, New York, 595 pp
- Cona A, Rea G, Angelini R, Federico R, Tavladoraki P (2006) Functions of amine oxidases in plant development and defence. *Trends in Plant Science* **11**, 80-88
- Costa G, Bagni N (1983) Effects of polyamines on fruit-set of apples. *HortScience* **18**, 59-61
- Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 355-381
- D'Orazi D, Bagni N (1987) *In vitro* interactions between polyamines and pectic substances. *Biochemical Biophysical Research Communication* **148**, 1159-1163
- Fan X, Mattheis JP, Fellman JK (1998a). A role for jasmonates in climacteric fruit ripening. *Planta* **204**, 444-449
- Fan X, Mattheis JP, Fellman JK (1998b) Responses of apples to postharvest jasmonate treatments. *Journal of the American Society for Horticultural Science* **123**, 421-425
- González-Aguilar GA, Buta JG, Wang CY (2001) Methyl jasmonate reduces chilling injury symptoms and enhances colour development of 'Kent' mangoes. *Journal of the Science of Food and Agriculture* **81**, 1244-1249
- Groppa MD, Benavides MP (2008) Polyamines and abiotic stress: recent advances. *Amino Acids* **34**, 35-45
- Hanfrey C, Franceschetti M, Mayer MJ, Illingworth C, Michael AJ (2002) Abrogation of upstream open reading frame-mediated translational control of a plant S-adenosylmethionine decarboxylase results in polyamine disruption and growth perturbations. *The Journal of Biological Chemistry* **277**, 44131-44139
- Janoudi A, Flore JA (2003) Effects of multiple applications of methyl jasmonate on fruit ripening, leaf gas exchange and vegetative growth in fruit trees. *Journal of Horticultural Science and Biotechnology* **78**, 793-797
- Khan AS, Singh Z (2007) Methyl jasmonate promotes fruit ripening and improves fruit quality in Japanese plum. *Journal of Horticultural Science and Biotechnology* **82**, 695-706
- Khan AS, Singh Z, Abbasi NA (2007) Pre-storage putrescine application suppresses ethylene biosynthesis and retards fruit softening during low temperature storage in 'Angelino' plum. *Postharvest Biology and Technology* **46**, 36-46
- King GA, Henderson KG, Lill RE (1987) Growth and anatomical and ultrastructural studies of nectarine fruit wall development. *Botanical Gazette* **148**, 433-455
- Kondo S, Tomiyama A, Seto H (2000) Changes of endogenous jasmonic acid

- and methyl jasmonate in apples and sweet cherries during fruit development. *Journal of the American Society for Horticultural Science* **125**, 282-287
- Kondo S, Setha S, Rudell DR, Buchanan DA, Mattheis JP** (2005) Aroma volatile biosynthesis in apple affected by 1-MCP and methyl jasmonate. *Postharvest Biology and Technology* **36**, 61-68
- Kondo S, Yamada H, Setha S** (2007) Effects of jasmonates differed at fruit ripening stages on 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase gene expression in pears. *Journal of the American Society for Horticultural Science* **132**, 120-125
- Kramer GF, Wang CY, Conway WS** (1991) Inhibition of softening by polyamine application in 'Golden Delicious' and 'McIntosh' apples. *Journal of the American Society for Horticultural Science* **116**, 813-817
- Kusano T, Berberich T, Tateda C, Takahashi Y** (2008) Polyamines: essential factors for growth and survival. *Planta* **228**, 367-381
- Kushad MM** (1998) Changes in polyamine levels in relationship to the double sigmoidal growth curve of peaches. *Journal of the American Society for Horticultural Science* **123**, 950-955
- Liu JH, Honda C, Moriguchi T** (2006) Involvement of polyamine in floral and fruit development. *Japan Agricultural Research Quarterly* **40**, 51-58
- Martinez-Romero D, Valero D, Serrano M, Burló F, Carbonell A, Burgos L, Riquelme F** (2000) Exogenous polyamines and gibberellic acid effects on peach (*Prunus persica* L.) storability improvement. *Journal of Food Science* **65**, 288-294
- Martin-Tanguy J** (1985) The occurrence and possible function of hydroxycinnamoyl acid amides in plants. *Plant Growth Regulation* **3**, 381-399
- Masia A, Zanchin A, Rascio N, Ramina A** (1992) Some biochemical and ultrastructural aspects of peach fruit development. *Journal of the American Society for Horticultural Science* **117**, 808-815
- Mattoo AK, Handa AK** (2008) Higher polyamines restore and enhance metabolic memory in ripening fruit. *Plant Science* **174** 386-393
- Mattoo AK, Sobolev AP, Neelam A, Goyal RK, Handa AK and Segre AL** (2006) Nuclear magnetic resonance spectroscopy-based metabolite profiling of transgenic tomato fruit engineered to accumulate spermidine and spermine reveals enhanced anabolic and nitrogen-carbon interactions. *Plant Physiology* **142**, 1759-1770
- Metha RA, Cassol T, Li N, Handa AK, Mattoo AK** (2002) Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life. *Nature Biotechnology* **20**, 613-618
- Mirdehghan SH, Rahemi M, Serrano M, Guilleán F, Martínez-Romero D, Valero D** (2007) The application of polyamines by pressure or immersion as a tool to maintain functional properties in stored pomegranate arils. *Journal of Agricultural and Food Chemistry* **55**, 755-760
- Pang XMi, Zhang ZY, Wen XP, Ban YM, Moriguchi T** (2007) Polyamines, all-purpose players in response to environment stresses in plants. *Plant Stress* **1**, 173-188
- Paksasorn A, Hayasaka T, Matsui H, Ohara H, Hirata N** (1995) Relationship of polyamine content to ACC content and ethylene evolution in Japanese apricot fruit. *Journal of the Japanese Society for Horticultural Science* **63**, 761-766
- Pérez AG, Sanz C, Richardson DG, Ollás JM** (1993) Methyl jasmonate vapor promotes β -carotene synthesis and chlorophyll degradation in 'Golden Delicious' apple peel. *Journal of Plant Growth Regulation* **12**, 163-167
- Perez-Vicente A, Martínez-Romero D, Carbonell A, Serrano M, Riquelme F, Guillén F, Valero D** (2002) Role of polyamines in extending shelf life and the reduction of mechanical damage during plum (*Prunus salicina* Lindl.) storage. *Postharvest Biology and Technology* **25**, 25-32
- Petkou JT, Pritsa TS, Sfakiotakis EM** (2004) Effects of polyamines on ethylene production, respiration and ripening of kiwifruit. *Journal of Horticultural Science and Biotechnology* **79**, 977-980
- Ravel S, Gakière B, Job D, Douce R** (1998) The specific features of methionine biosynthesis and metabolism in plants. *Proceedings of the National Academy of Sciences USA* **95**, 7805-7812
- Rohwer CL, Erwin JE** (2008) Horticultural application of jasmonates. *Journal of Horticultural Science and Biotechnology* **83**, 283-304
- Rudell DR, Mattheis JP** (2008) Synergism exists between ethylene and methyl jasmonate in artificial light-induced pigment enhancement of 'Fuji' apple fruit peel. *Postharvest Biology and Technology* **47**, 136-140
- Schmidt DD, Baldwin IT** (2006) Transcriptional responses of *Solanum nigrum* to methyl jasmonate and competition: a glasshouse and field study. *Functional Ecology* **20**, 500-508
- Srivastava A, Chung SH, Fatima T, Datsenka T, Handa AK, Mattoo AK** (2007) Polyamines as anabolic growth regulators revealed by transcriptome analysis and metabolite profiles of tomato fruits engineered to accumulate spermidine and spermine. *Plant Biotechnology* **24**, 57-70
- Tassoni A, Antognoni F, Battistini ML, Sanvido O, Bagni N** (1998) Characterization of spermidine binding to solubilized plasma membrane proteins from zucchini hypocotyls. *Plant Physiology* **117**, 971-977
- Torrighiani P, Bregoli AM, Ziosi V, Scaramagli S, Ciriaci T, Rasori A, Biondi S, Costa G** (2004) Pre-harvest polyamine and aminoethoxyvinylglycine (AVG) applications modulate fruit ripening in Stark Red Gold nectarines (*Prunus persica* L. Batsch). *Postharvest Biology and Technology* **33**, 293-308
- Torrighiani P, Bregoli AM, Ziosi V, Costa G** (2008) Molecular and biochemical aspects underlying polyamine modulation of fruit development and ripening. *Stewart Postharvest Review* **4**, 1-12
- Trainotti L, Bonghi C, Ziliotto F, Zanin D, Rasori A, Casadoro G, Ramina A, Tonutti P** (2006) The use of microarray μ PEACH1.0 to investigate transcriptome changes during transition from pre-climacteric to climacteric phase in peach fruit. *Plant Science* **170**, 606-613
- Valero D, Martínez-Romero D, Serrano M** (2002) The role of polyamines in the improvement of the shelf life of fruit. *Trends in Food Science and Technology* **13**, 228-234
- Vezzulli S, Civardi S, Ferrari F, Bavaresco L** (2007) Methyl jasmonate treatment as a trigger of resveratrol synthesis in cultivated grapevine. *American Journal of Enology and Viticulture* **58**, 530-533
- Wang SY, Bowman L, Ding M** (2008) Methyl jasmonate enhances antioxidant activity and flavonoid content in blackberries (*Rubus* sp.) and promotes anti-proliferation of human cancer cells. *Food Chemistry* **107**, 1261-1269
- Wasternack C** (2007) Jasmonates, an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Annals of Botany* **100**, 681-697
- Yu YB, Yang SF** (1979) Auxin-induced ethylene production and its inhibition by aminoethoxyvinylglycine and cobalt ion. *Plant Physiology* **64**, 1074-1077
- Ziosi V, Scaramagli S, Bregoli AM, Biondi S, Torrighiani P** (2003) Peach (*Prunus persica* L.) fruit growth and ripening: transcript levels and activity of polyamine biosynthetic enzymes in the mesocarp. *Journal of Plant Physiology* **160**, 1109-1115
- Ziosi V, Bregoli AM, Bonghi C, Fossati T, Biondi S, Costa G, Torrighiani P** (2006a) Transcript levels of ethylene perception and biosynthesis genes as altered by putrescine, spermidine and aminoethoxyvinylglycine (AVG) during the course of ripening in peach fruit (*Prunus persica* L. Batsch). *New Phytologist* **172**, 229-238
- Ziosi V, Bregoli AM, Bonghi C, Rasori A, Biondi S, Costa G, Torrighiani P** (2006b) Jasmonates delay ripening by interfering with ethylene biosynthesis and perception and with polyamine accumulation in peach fruit. In: Ramina A, Chang C, Giovannoni J, Klee H, Perata P, Woltering E (Eds) *Advances in Plant Ethylene Research*. Proceedings of the 7th International Symposium on the Plant Hormone Ethylene (2006), Springer, Dordrecht, The Netherlands, pp 109-110
- Ziosi V, Bonghi C, Bregoli AM, Trainotti L, Biondi S, Setha S, Kondo S, Costa G, Torrighiani P** (2008) Jasmonate-induced transcriptional changes suggest a negative interference with the ripening syndrome in peach fruit. *Journal of Experimental Botany* **59**, 563-573
- Ziosi V, Bregoli AM, Fregola F, Costa G, Torrighiani P** (2009) Jasmonate-induced ripening delay is associated to up-regulation of polyamine levels in peach fruit. *Journal of Plant Physiology* **166**, 938-946