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Combined Effects of Fungicides and Thermotherapy on Post-Harvest Quality of Horticultural Commodities

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1. Introduction

Production, market and consumption of fresh fruit and vegetables (Fig. 1) are continuously increasing in the world (FAOSTAT, 2011). This increase is not only linked to the growth of the world population and to the consequent demand of food but also to the continuous improving of the food quality in the emerging countries. Immediately after the satisfaction of the energy needs, in fact, more lipids are introduced in the diet with the aim of improving the organoleptic quality of the food. Subsequently, a wide variety of ingredients, including fresh fruit and vegetables are requested to integrate the daily diet. Cereals are absolutely fundamental as they provide energy, proteins, lipids and minerals. However, fibre, minerals, vitamins, sugars, acids, aminoacids, and other compounds, like polyphenols and other antioxidants of particular importance because of their nutritional and nutraceutical value are provided by fresh fruits and vegetables (Schreiner & Huyskens-Keil, 2006).

In spite of the large variability of horticultural commodities marketed today in the world, not all the regions of the globe showed possibilities to give high yields of many crops. Often geographical reasons made impossible the production of any commodity, like in the extreme latitude North and South, or in arid and desert areas. Technology advances may contribute to extend the growing area of some crops, but not always there is a real economic convenience in this effort. Furthermore, in other lands poverty and lack of professional abilities make difficult cultivation even when the natural environment is potentially favourable. For these reasons the international market of horticultural commodities is very big and diversified. The most developed countries manage the highest part of exchanges, while a part of producer countries showed an increasing activity with the aim of taking a part of the international market management due to quality of commodities and competitive prices. Some of these "emerging" countries have also an internal market absolutely wide, and an increasing demand which is the market base to export commodities of excellent and selected quality. This is the case of Brazil, South Africa, Mexico, India, and China. Some other advanced countries have in the export of horticultural commodities a point of excellence of their economy, like Spain, Italy, Turkey, Israel, and Greece. Moreover, other "emerging" countries are small producers and have a small internal market, but have found a good specialization in the export of high value horticultural commodities thus obtaining an interesting added value from this activity: like Cyprus, Chile, Cuba, Costa Rica and Morocco.

The big quantity of fresh horticultural commodities that are the object of import/export on the international markets, but also on the big internal markets, is characterized by quality and nutritional properties that should be maintained until the final consumer (Kader, 2003). Many difficulties operate against this objective. The first is the physiological weight loss of horticultural commodities determined by both water loss, due to transpiration of living plant tissues, and by dry matter loss due to product respiration. This weight and value loss may be comprised between 10 and 20%. Other losses are determined by some physiological disorders like the chilling injury which can occur during and after the cold storage. The cold storage is often a need in order to preserve the product quality by minimizing the physiological losses and the mould decay. The losses for chilling injury are comprised between 10 and 15%, mainly for tropical and subtropical fruits and vegetables. Finally, the biggest losses are caused by specific postharvest diseases. Fungi and other microorganisms may be the responsible of 15-25% decay of horticulture commodities during storage and transport (Barkai-Golan, 2001).

Since the first experiences of the fruit and vegetable postharvest export industry, fungicide treatments have been applied to horticultural commodities in order to reduce mould decay in the best effective way. However, approved fungicides for postharvest treatments have been always a small number of compounds because of the short period of tolerance between application and product consumption. Severity in the control of fungicides residues is also usual in technological advanced importing countries. Moreover, the amounts of fungicides applied to horticultural commodities during postharvest process are small respect to the applied on the field, and the cost for the authorization of a new product is very high, thus determining low interest in the development of new fungicides by the specialized chemical industries (Narayanasamy, 2006).

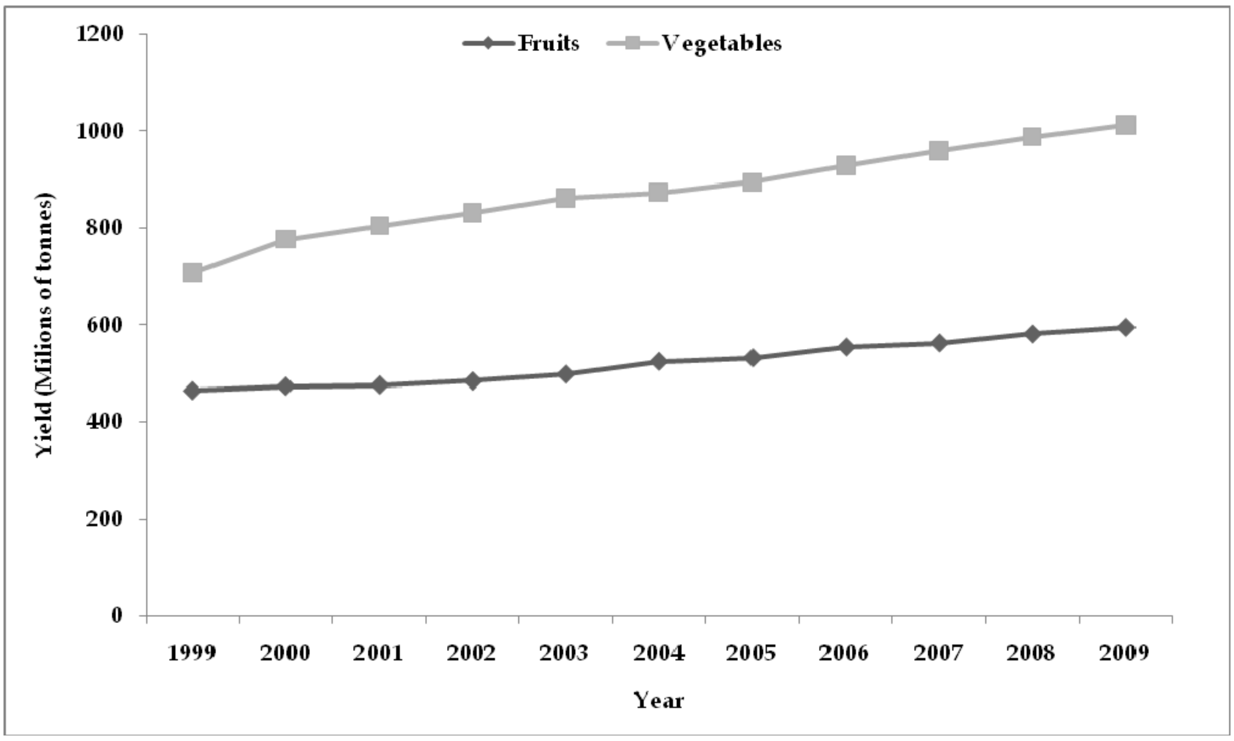

Fig. 1. World yield of fruits and vegetables in the last decade (data source: FAOSTAT 2011).

As a consequence of the repeated and exclusive use of the few admitted fungicides for postharvest treatments of fresh horticultural commodities in a very limited space like the processing and storage packing houses, many resistant strains for these fungicides of the main pathogens have been widely detected in the last decades.

The urgency to find alternative technologies of control against postharvest pathogens of horticultural commodities is clearly evidenced and sustained by the following arguments:

- the growth of the horticultural commodity quantity marketed and submitted to import/export movements;
- the increased risk to spread dangerous pests and pathogens with the fruits and vegetables shipped all over the world;
- the detection and isolation every day more frequent of strains of the main postharvest pathogens resistant to the commonly used fungicides;
- the claimed exigency of consumers and of importing countries to have foods free from any chemical compound dangerous for the human safety;
- the need to have more fungicides as possible alternatives in postharvest treatments;
- the possibility to increase fungicide effectiveness by combination with thermotherapy and with other friendly chemical or physical means generally recognized as safe (GRAS).

2. The thermotherapy of horticultural commodities

The use of the heat to control some postharvest citrus pathogens was experimented in the first decades of the past century (Mulas & Schirra, 2007). The first experiment cited in the literature was the postharvest use of hot water and hot sodium hydroxide solutions to control citrus moulds. Subsequently, the use of the thermotherapy was also widely applied against parasites of horticultural commodities (Barkai-Golan & Phillips, 1991; Lurie, 1998; Schirra et al., 2000a; Shellie & Robert, 2000; Fallik, 2004; Fallik & Lurie, 2007).

In the 70th years a wide availability and diffusion of fungicides made less rentable the use of heat in post-harvest treatments which always were linked to supplementary energy costs (Schirra, 2005). However, it was just in the years of widest diffusion of synthetic fungicides that the advantages to combine postharvest treatments with warming of active solutions were observed. This improved efficiency was associated to a better distribution, penetration and cuticle diffusion of fungicides in the commodity tissues (Cabras et al., 1999; Schirra et al., 2002a). Usually the cuticle showed a relatively low permeability to most agricultural chemicals (Baur & Schönherr, 1995; Schreiber & Schönherr, 2009) but we have to consider the presence of cracks or other breaks in the cuticle surface that increases with fruits maturation (Faust & Shear, 1972; Freeman et al., 1979; Barthlott, 1990; Bianchi, 1995). These cracks are important for the fungicide uptake in plant tissues but, when the application is combined with thermotherapy, there is also a partial fusion and distribution of cuticular waxes that cover these cracks and reduce the ways of fungal infection (Gleen et al., 1985; El-Otmani et al., 1989; Roy et al., 1994; 1999; Bally, 1999). Many studies have demonstrated this evidence in different commodities, including citrus (Schirra & D’hallewin, 1997; Schirra et al., 1998a; Porat et al., 2000), cactus pear (D’hallewin et al., 1999; Schirra et al., 1999), sweet pepper (Fallik et al., 1999), and melon (Fallik et al., 2000).

Evaluations and experiments of thermotherapy combination have been reported on the application of a widely used fungicide to control moulds in citrus fruit (Eckert & Ogawa, 1985; Eckert & Eaks, 1988). Treatments with Sodium ortho-phenylphenate (SOPP) were performed after warming at 40 °C to control citrus moulds on 'Pineapple' and 'Valencia' oranges (Hayward & Grieson, 1960). The same fungicide was used at a concentration of 2%, with 1% hexamine and 0.2% sodium hydroxide in water at 22.8 and 37.8 °C. In this case the cold solution was more effective to control mould decay than the heated solution because of the phytotoxic effect of the SOPP (McCornack & Hayward, 1968).

Heated and unheated fungicides were tested on apples to control blue mould development (Spalding et al., 1969).

With the aim of controlling *Monilinia fructicola,* a solution of 2,6-diclhoro-4-nitroaniline was positively used at 51.5 °C for one and a half minute dip on peaches, plums, and nectarines (Wells & Harvey, 1970). These results were reconsidered and the research on thermotherapy was newly promoted in the 80th years (Carter, 1981a) due in part to the new cultural approach to the environment problems, the higher care for the human health and the difficulties to accept the use of fungicides and other chemical controlling pests largely used in the food industry (Schirra, 2005).

Actually we have to consider that few postharvest fungicides (Tomlin, 1997; Adaskaveg & Förster, 2010) are admitted to postharvest treatments of horticultural commodities and that their effectiveness is largely compromised by the natural selection of resistant strains of the main postharvest pathogens, as a consequence of their repeated use (Schmidt et al., 2006). For this reason, the research of alternatives to chemical pesticides for the control of postharvest disorders is considered as a prioritary objective for many researcher involved in the test of many methods: thermotherapy, use of radiations, gas and vapour, use of microbiological resources (like antagonist or toxic strains), use of chemical compounds of not toxic salts and compounds generically recognized as safe (GRAS).

2.1 Applications of thermotherapy

The thermotherapy is possible thank to the transfer of thermal energy to the horticultural commodity by means of a fluid mean which can be liquid, water vapour or air saturated of humidity. Hot dip are made in water with temperatures ranging from 43 to 53 °C and time of dipping comprised between 1-3 minutes and 2 hours (Ben-Yehoshua et al., 2000; Fallik, 2004). A different kind of dip treatment is the brushing of horticultural commodities under a hot water flow. Water temperature in this case are higher than in the case of hot dip, ranging between 48 and 60-63 °C during shorter periods of 15-30 seconds until 1 minute.

Treatments with air or hot vapour showed some technical difficulties and need more time but often their effectiveness is more prolonged with respect to hot dip (Mulas & Schirra, 2007).

As a further possibility among others, applications of radiofrequency waves may be useful for thermotherapy of horticultural commodities as actually in the wood industry or for the treatment of cereals (Johnson et al., 2003).

2.2 Control of physiological disorders

Fruits and vegetables of tropical and subtropical origin like citrus are particularly sensitive to chilling injury. Also this physiological disorder may be reduced or avoided by means of the thermotherapy, like the exposition of the commodities to hot air at 37 °C during 3 days under saturated humidity (Lafuente & Zacarias, 2006), or by mean of applications of hot dip

(Wild & Hood, 1989; McDonald et al., 1991; Wang, 1993; Gonzales-Aguilar et al., 1997; Mulas, 2006). More indications are available in order to control chilling injury of horticultural commodities of the temperate regions, like apples, by means of the thermotherapy (Lurie, 1998; Mulas & Schirra, 2009).

The effectiveness of thermotherapy to control oxidative stress in chilled fruits seems also linked directly to the enhanced activity of enzymes controlling antioxidant responses (Sala, 1998; Sala & Lafuente, 1999; 2000).

2.3 Control of pests

Many markets require horticultural commodities absolutely free of pesticide residues but at the same time also free from eggs, larvae and adults of insects. Also in this direction, alternative methods to the use of chemical fumigants, like methyl-bromide (forbidden in many countries), have been developed. Optimal times and heating temperatures for disinfestations depend on species and cultivars. For citrus, temperature of 44 °C for 90 minutes or 46 °C for 50 minutes are indicated as effective, but it is true that not all the citrus cultivars withstand these treatments. Thus, symptoms of senescence have been observed after this kind of treatments in bloody and ‘Valencia late’ oranges (Mulas et al., 2001; Schirra et al., 2004; 2005a).

Good results have been obtained against some coleoptera infesting dates by heating them at 55 °C for 2 hours and 30 minutes (Rafaeli et al., 2006).

2.4 Direct control of postharvest pathogens

Hot dip is effective controlling main postharvest pathogens of horticultural commodities both as a consequence of the fruit surface washing leading to reduced inoculums, as well as because of the thermal inactivation of the microorganisms. Hot dips and high temperature conditioning were effective to improve shelf quality of late crop cactus pear fruit (Schirra et al., 1996a).

Furthermore, we have to consider the possibility that thermotherapy may stimulate the biosynthesis or elicitation of endogenous compounds having fungicide effects (Lurie, 1998; Schirra et al., 2000a).

Evidence of these effects on fruit surface have been showed in a research carried out on 'Montenergrina' mandarins (Montero et al., 2010), where hot dip treatments at 60 °C with brushing significantly reduced the number of tangerines affected by decay.

In a recent study, hot water dip at 48 °C for 12 or 6 minutes showed good control of postharvest brown rot on 'Roig' peach and 'Venus' nectarine, without visual symptoms of heat damage and significant loss of fruit quality (Jemric et al., 2011). The elicitation of compounds involved in the plant defence as a consequence of hot air treatments at 38 °C during 24-72 hours was further demonstrated on tomato cherry fruit by means of higher lignin deposition, and higher activation of phenylalanine ammonia-lyase and β-1,3-glucanase (Zhao et al., 2009; 2010).

3. Combination of thermotherapy with fungicides

In spite of the beneficial effects of thermotherapy to the control of microbial postharvest disorders of fresh horticultural commodities, this treatment is not able to have the same effectiveness of fungicides, particularly when the marketing is made after a period more or less long of storage (Schirra, 2005; Mulas, 2008). In some countries, thermotherapy is applied to control mould decay in commodities from organic cultivation or when the postharvest fungicide treatment is completely forbidden (Fallik, 2004). In other cases, the chemical defence is always of fundamental importance against mould decay and there is not a convenience to use other methods (Dezman et al., 1986; Papadopoulou-Mourkidou, 1991).

3.1 Old fungicides

After the first experiences in the 60th years, many investigations have been promoted in the 70th and 80th years, but a new start of researches on old fungicides was observed in the 90th.

Mango decay was controlled by combination of hot dips with Benomyl (Spalding & Reeder, 1972; 1978). Brown rot and rhyzopus of peaches, nectarines and stone fruits were controlled by different combination of Benomyl or other fungicides and thermotherapy (Smith, 1971; Wells & Gerdts, 1971; Wells, 1972; Jones & Burton, 1973; Smith & Anderson, 1975; Wang & Anderson, 1985).

The control of rots of guavas was tested by application of heated Benomyl and Guazatine dips (Wills et al., 1982). Rotting and browning of lytchee fruit were controlled by hot benomyl and plastic film (Scott et al., 1982; Wong et al., 1991).

New experiments were carried out to demonstrate the synergistic effect of heat and sodium ortho-phenylpenate to inactivate *Penicillium* spores and suppress decay in citrus fruits (Barkai-Golan & Appelbaum, 1991). The efficacy of hot water and Carbendazim treatments was tested against the brown rot of apple (Sharma & Kaul, 1990).

3.2 Thiabendazole and Imazalil

The most used fungicides in postharvest of horticultural commodities are Imazalil and Thiabendazole. Both are synthetic compounds with different mode of action that can be applied in waxes or water (Brown et al., 1983; Brown, 1984). Imazalil is very effective against the green mould (*Penicillium digitatum*), including benzimidazole-resistant strains, while Thiabendazole is effective to a wide range of pathogens and often may have beneficial effects also against chilling injury (Schiffman-Nadel et al., 1972; 1975; Brown & Dezman, 1990; McDonald et al., 1991; Schirra et al., 2000b).

Many studies demonstrate that the efficacy of Imazalil and/or Prochloraz increases when applied in hot water in a number of fruits such as mango (Spalding & Reeder, 1986; Johnson et al., 1990; Prusky et al., 1990; Dodd et al., 1991; Coates et al., 1993; McGuire & Campbell, 1993; Waskar, 2005); melons (Carter, 1981b; Mayberry & Kartz, 1992), taro corms (Quevedo et al., 1991), red tamarillos (Yearsley et a., 1988), and in citrus fruit (Ansari & Feridoon, 2008).

That is because of the best cuticle and tissue penetration by mean of active compounds (Schirra et al., 2002a; 2008a). Wide demonstration of these effects has been reported in the case of the Imazalil and Thiabendazole use against *Penicillium* mould of citrus (Mulas & Schirra, 2007; Dore et al., 2009). The two fungicides resulted effective in some cases with very low concentration, up to 50 mg $\cdot L^{-1}$, if the application was made in hot water (50 °C) (Schirra & Mulas, 1993; 1994; 1995a; b; c; Schirra et al., 1995; 1997a).

Treatments of citrus with Imazalil at 490 mg·L^{-1}, in water at 37.8 °C resulted more effective in mould control than in wax mixture at 4200 mg·L^{-1} at ambient temperature (Smilanick et al., 1997).

The residue control of Imazalil in lemons after applications at 50 °C of concentrations from 250 to 1500 mg·L^{-1} demonstrated that fruit submitted to hot dip contained about 4.5-fold higher fungicide concentration (Schirra et al., 1996b). Good results were obtained with hot dip in Imazalil mixtures of lemons (Schirra et al., 1997b) and 'Marsh' grapefruits (Schirra et al., 1998b).

Thiabendazole residues are dependent on pH of solutions (Wardowski et al., 1974), not influenced by duration of the treatment (Cabras et al., 1999), and correlated to the amount of fungicide applied (Schirra et al., 1998c)

Synergic effect of thermotherapy with fungicide application was not always so clear, and in the past there is evidence of some negative combination of the two treatments, like in the case of 'Tarocco' oranges when the preharvest treatment with Thiabendazole was effective in the postharvest control of *Penicillium* moulds and against chilling injury, but the combination with a curing treatment at 37 °C during 48 hours resulted favourable to the decay by *Phytophthora citrophtora* (Schirra et al., 2002a).

Positive effects of Thiabendazole in combination with hot dip have been confirmed by treatments on 'Eureka' lemons at 0, 25, 50 e 100 μg·mL^{-1} in water containing 200 μg·mL^{-1} of sodium hypochlorite and 0.2 ml·L^{-1} of Triton X-100, at temperature of 16, 27, 38, or 49 °C after artificial inoculation with a Thiabendazole resistant strain of *Penicillium digitatum* (M6R) (Smilanick et al., 2006a). In fact, mould control by only dip temperature was of 50% at 49 °C, while the addition of only 100 μg·mL^{-1} of Thiabendazole was effective for an almost total control of the pathogen.

A further confirmation of the synergic effect between postharvest low dose treatment with Thiabendazole and warming of the dip solution at 52 °C was observed with the control of pathogens and, partially, of the chilling injury in cactus fruit of the 'Gialla' cultivar (Schirra et al., 2002b).

In tamarillos, Imazalil was effective against *Collectotricum gleosporioides* or *C. acutatum* both in water and in wax if fungicide application followed a hot dip treatment in water at 50 °C for 10 minutes (Yearsley et al., 1987).

Good results were also recorded for the control of *Penicillium* mould and of chilling injury in citrus fruits by the fungicide Imazalil when this compound was used after molecular complexation with β-cyclodestrine at 100 mg·L^{-1} in water dip at 50 °C (Schirra et al., 2002c). Different works made with other fungicides demonstrate the effectiveness of β-cyclodestrine since it is a carrier of active molecules that prevents their degradation (Szejtli, 1988; Kenawy et al., 1992; Lezcano et al., 2002).

3.3 New fungicides

Most of the positive effects of synergy between thermotherapy and low dose applications of fungicides has been confirmed with the experimental use of a new generation of fungicides so called "natural mimetic"(Gullino et al., 2000; Ragsdale, 2000; Leroux, 2003). These compounds are also defined like generally recognized as safe (GRAS) with respect to the previously used fungicides. They showed higher effectiveness at low doses, a more favourable toxicological and eco-toxicological profile and different mechanisms of action with respect to the old generation of fungicides (Heye et al., 1994; Errampalli & Crnko, 2004). Therefore, these new fungicides may be very useful as an alternative to traditional fungicides which are ineffective against resistant strains of pathogens (Schirra, 2005).

Among others it is important to consider the experiences realized with the strobilurine-like compounds Azoxystrobine and Trifloxistrobine (Margot et al., 1998; Reuveni, 2000; 2001; Barlett et al., 2002; Wood & Hollomon, 2003; Schirra et al., 2006), which have their site of action in the fungal mitochondrion and are quickly destroyed in soil and groundwater (Sudisha et al., 2010). Preharvest application of Azoxystrobine were effective to control *Alternaria alternata* in mandarin cultivars 'Minneola' and 'Nova' (Oren et al., 1999) and citrus scab and melanose in seedlings of rough lemon and grapefruit (Bushong and Timmer, 2000).

Azoxystrobine in postharvest was applied to 'Star Ruby' grapefruits and oranges by 3 minutes dip at 50 °C and low concentration (50 mg $\cdot$L^{-1}) (Schirra et al., 2002d; 2010). The long ability of Azoxystrobine residues to remain constant during cold storage and in darkness was confirmed by other studies on apples (Ticha et al., 2008) and peppers (Garau et al., 2002), while rapid decline of residues was observed with preharvest treatments (Angioni et al., 2004). However, Azoxystrobine showed good natural decay control but was less effective against artificially inoculated *P. digitatum*, while Trifloxistrobine was highly effective against blue and green mould after inoculation and in association with hot dip at 50 °C for 3 minutes (Schirra et al., 2006).

Further experiences were made with Fludioxonil (synthetic analogous of pyrrolnitrine), which after fruit inoculation with *Penicillium* provided the same results as Imazalil at 100 mg $\cdot$L^{-1} in water dip at 50 °C and 400 mg $\cdot$L^{-1} at 20 °C (Schirra et al., 2005b), and with Pyrimethanil (anilinepyrimidine) that resulted effective at 400 mg $\cdot$L^{-1} at 20 °C or 100 mg $\cdot$L^{-1} at 50 °C against *P. digitatum* and *P. italicum* (D'Aquino et al., 2006). Pyrimethanil inhibits elongation of mycelium and the secretion of cell wall degradation enzymes (Daniels & Lucas, 1995; Milling & Richardson, 1995; Rosslenbroich & Stuebler, 2000; Sholberg et al., 2005; Kanetis et al., 2008a). Pyrimethanil increased notably their effectiveness when coupled with thermotherapy (D'Aquino et al., 2006; Smilanick et al., 2006b).

Cyprodinil was positively tested in combination with hot water dip against apple moulds (Errampalli & Brubacher, 2006) and inoculated *Penicillium digitatum* moulds on 'Valencia' oranges (Schirra et al., 2009a).

The possibility to control many postharvest pathogens has been investigated on a wide range of horticultural commodities (Smilanick et al., 2006b; Zhang, 2007; Kanetis et al., 2008a; Montesinos-Herrero & Palou, 2010) and recently Azoxystrobine, Fludioxonil and Pyrimethanil have been authorized for postharvest treatments in U.S.A. (Kanetis et al., 2007; Förster et al., 2007).

The mode of action of Fludioxonil is that of a mutagen-activated protein chinase pathway that stimulates glycerol synthesis (Kanetis et al., 2008b).

Recently, fludioxonil was also positively tested in association with thermotherapy on mango (Swart et al., 2009), pomegranates (Palou et al., 2007; D'Aquino et al., 2009), apple (Errampalli, 2004; Errampalli et al., 2005), citrus (Schirra et al., 2005), nectarines, apricots and peaches (D'Aquino et al., 2007), and 'Precoce di Fiorano', 'Coscia' and 'Spadona estiva' summer pears after inoculation with *Penicillium expansum* and *Botrytis cinerea* (Schirra et al., 2008b; 2009b).

Fig. 2. Stored mandarins treated with Imazalil at 100 mg $\cdot L^{-1}$ and 50 °C and 400 mg $\cdot L^{-1}$ at 20 °C against *P. italicum* (Schirra et al., 2005b; courtesy of Dr. Mario Schirra).

Fig. 3. Stored mandarins treatment with pyrimethanil at 100 mg $\cdot L^{-1}$ and 50 °C against *P. italicum* (D'Aquino et al., 2006; courtesy of Dr. Mario Schirra).

Fig. 4. Wounding of ‘Coscia’ pears before inoculation with *Penicillium expansum* (upper), apparatus for water dip at 50 °C (middle), and mould development after shelf life (above) (Schirra et a., 2008b; pictures of the author).

Fig. 5. Stored ‘Coscia’ pears after inoculation with *Penicillium expansum* (upper), water dip at 50 °C (middle), and treatment with fludioxonil at 100 mg $\cdot L^{-1}$ and 50 °C (above) (Schirra et a., 2008b; pictures of the author).

4. GRAS compounds

Researchers have focused their interest on GRAS compounds in order to find alternatives to traditional fungicides or to enhance their effectiveness by means of synergistic effects when combined with thermotherapy. Among others, it is interesting to point out calcium chloride preharvest applications in combination with 2,4-D, hot dip and fungicides on 'Satsuma' mandarins (Yildix et al., 2005); the ethanol used for the control of *B. cinerea* after inoculation on table grape (Karabulut et al., 2004; Gabler et al., 2005); the acetic acid as an alternative to ethanol or water vapour to control *B. cinerea* on kiwi fruit (Lagopodi et al., 2009); the sodium carbonate and bicarbonate, which were effective against *Penicillium* mould in citrus fruits dipped for 150 seconds in water at 45 °C containing a 3% of the salt (Palou et al., 2001). This is also the case of the bicarbonate, which resulted useful on citrus fruit in order to increase the effectiveness of Imazalil to inhibit germination of *P. digitatum* (Smilanick et al., 2005), and of Thiabendazole against the resistant strain of *P. digitatum* M6R in combination with dip at 49 °C during 60 seconds and addition of sodium hypochlorite at 200 $\mu g \cdot mL^{-1}$ (Smilanick et al., 2006). Good results have been also obtained by application of Imazalil or Thiabendazole in combination with potassium sorbate (Smilanick et al., 2008).

Potassium phosphite (2 $mg \cdot mL^{-1}$) in combination with hot dip at 50 °C for 3 minutes induced a three-fold reduction in blue mould incidence and was as effective as Thiabendazole after six months of storage at 2 °C of 'Elstar' apples (Amiri & Bompeix, 2011).

The positive effect of carbonate and bicarbonate addition to solutions of Thiabendazole used to control *P. digitatum* was demonstrated also on clementine fruits, 'Nova' mandarins, 'Valencia late' oranges with a higher penetration of the fungicide in the fruit tissues (Schirra et al., 2008c). 'Montenegrina' tangerines were exposed to postharvest thermotherapy and sodium carbonate and bicarbonate treatments in combination with carnauba wax application, which resulted in fruit protection against mould decay (Montero et al., 2010).

On 'Satsuma' mandarins, the combination of thermotherapy and fungicides was effective against resistant strains of pathogens, and the combination of thermotherapy with antagonist microorganisms was effective against the *Rhizopus* decay on strawberry (Zhang et al., 2005). On the other hand, the synergic effect of thermotherapy with the use of antagonist strains of yeasts and sodium bicarbonate was demonstrated against *Colletotrichum acutatum* and *P. expansum* in stored apples (Mulas & Schirra, 2007), as well as of thermotherapy in combination with *Cryptococcus laurentii* against *P. italicum* and *Rhizopus stolonifer* on peach, and with *Rhodotorula glutinis* against *P. italicum* on pear storage (Zhang et al., 2007; 2008).

Prevention of spoilage caused by fungi in cherry tomato was provided by heat treatment at 38 °C (24-72 hours) followed by *Pichia guilliermondii* application (Zhao et al., 2009; 2010). Other results showed that the combined application of hot air at 38 °C for 36 hours and the same yeast antagonist *Pichia guilliermondii* was effective in the control of postharvest anthracnose rot of loquat fruit (Liu et al., 2010).

The residue determination of pesticides, or of other chemicals used in combination with thermotherapy, is an essential condition to guarantee treatment effectiveness avoiding to overcome levels of tolerance. This is the only way to maintain the safety of horticultural commodity, which is associated with the absence of chemical residues (Schirra, 2005).

5. Main effects on quality of commodities

In spite of the numerous applications of thermotherapy to many horticultural commodities, there are generalized recurrences of not complete control of mould decay if the treatments are limited to the physical means (Lurie, 2006). At the present state of knowledge, it seems more appropriate the use of thermotherapy as a synergic tool of the available fungicides and GRAS compounds (Schirra, 1995; Mulas & Schirra, 2007).

Among quality traits is important to point out that thermotherapy may build-up volatile compounds as shown in 'Tarocco' (Schirra et al., 2002b) and other bloody oranges (Mulas et al., 2001; Schirra et al., 2004; 2005a). Increases in endogenous ethanol and acetaldehyde production that change fruit taste have been reported in this fruits, but studies for a complete definition of gas exchange during and after thermotherapy are in a phase of development (Mulas et al., 2004; 2006; 2008a; 2008b).

5.1 Contra-indications

Many experiments of thermotherapy were designed to optimize the protocol to avoid decay or physiological disorders, but damages derived from this kind of treatments have been observed (Lurie, 2006). Negative effects have been described on apple fruit after brushing with water at 60 or 65 °C for 15 seconds (Fallik et al., 2001). Heat-damages manifested as increased electrolyte leakage from biological membranes and surface browning may occur in cactus pear fruit (*Opuntia ficus-indica* Miller L.) after brushing with water at 65-70 °C for 10-30 seconds (Dimitris et al., 2005). Negative effects of thermotherapy were also observed on strawberry (Wszelaki & Mitcham, 2003). Short hot-water rinsing and brushing treatment for 20 seconds at 55, 60, and 65 °C significantly reduced the epiphytic microbial population on fruit surface of strawberry cultivar 'Feng xiang', as well as decay development and weight loss (Jing et al., 2010). However, about 60% of the fruit treated at 65 °C showed heat damages.

The effects of treatment temperatures within the range from 20 to 75 °C were studied on 'Navelate' oranges after dip during 150 seconds (Palou et al., 2001). Any negative effect was recorded until 45 °C, while 17 and 28% of fruit treated at 53 or 55 °C showed slight or medium symptoms of heat damages on 100% of fruit surface.

The contemporary evolution of chilling injury and heat damage has been described in 'Satsuma' mandarins, as well as the involvement of antioxidant enzymes, vacuolar ATPase, and pyrophosphatase (Ghasemnezhad et al., 2007).

Heat damages may produce symptoms in the internal tissues of different commodities even in total absence of any external alteration. This is the case of the internal browning, which was observed in avocados, citrus, peaches, nectarines, and lytchees (Zhou et al., 2002; Follet & Sanxter, 2003; Lurie, 2006). Other symptoms, like low pulp colour evolution, anomalous softening, lack of starch hydrolysis, and development of internal cavities have been observed on mangoes and papaya (Jacobi et al., 2001; Lurie, 2006). Lytchee fruits of the cultivar 'McLean's Red' after water dip at 50 °C for 2 minutes or water dip at 55 °C for 1 minute showed superficial scald, while the treatment at 60 °C for 1 second was less harmful (Sivakumar & Korsten, 2006). Dragon fruits (*Hylocereus undatus*) were tolerant to hot air disinfestations treatments until a temperature of 46.5-48.5 °C for 20 minutes as measured in the fruit centre. Because the lack of significant differences for bracteas and peduncle turgidity, fruit general appearance and presence of mould decay, peel colour and pulp firmness, total soluble solids concentration, acidity, taste and pulp brightness, fruit quality was kept immediately after the treatment, after 4 weeks storage at 5 °C in propylene bags, and after the shelf life period at 20 °C (Hoa et al., 2006).

Some field variables, like seasonal temperatures and rainfall may influence the thermotherapy effectiveness, particularly in those commodity very sensitive to chilling injury or to heat scald like citrus fruit. Some studies demonstrate thermotherapy effectiveness in the control of chilling injury, but also that the same treatment may be harmful depending on the harvest date (Schirra et al., 1997; Lafuente et al., 2005; Lafuente & Zacarias, 2006).

5.2 Effects on maturation and senescence

Postharvest thermotherapy treatments slow down maturation of climacteric fruits (Fallik, 2004; Lurie, 2006). Ethylene biosynthesis inhibition by heat treatments slows pulp softening and favours low colour and aromatic compound development in apples and kiwis treated at 38 °C, while the treatment at 39 °C for 90 minutes slow down colour development in tomatoes (Ali et al., 2004).

Investigations on ‘Caldesi 2000’ nectarines and ‘Royal Glory’ peaches showed that water dip at 46 °C reduced pulp softening of fruits sealed in thin polyethylene bags and stored at 0 °C for one or two weeks (Malakou & Nanos, 2005). This effect is the result of the combination of hot water treatment, modified atmosphere application and package, particularly in white pulp nectarines, which maintain functional cell membranes because cell wall hydrolytic enzyme inactivation, mainly polygalacturonase. Hot air treatment may also change the organoleptic characteristics of peaches ‘Dixiland’ by decreasing total acidity and increasing red pigments in pulp and peel (Budde et al., 2006).

Usually the effects of postharvest heat treatments are reversible, if the application is not too long, and then the physiological damage is avoided. This is the case of the tomatoes treated with water at 42 °C that showed regular biosynthesis of aromatic compounds and of lycopene (Mulas & Schirra, 2007)

High temperature may induce temporary inhibition of polygalacturonase in mango and tomato, as well as low activity of other enzymes involved in softening. Changes in other characters linked to maturation may be reduced in non-climacteric fruits such as strawberries with slow colour development and pulp softening (Lurie, 2006), which may be associated with low acidity (Vicente et al., 2002).

Water dip at 45 °C improves strawberry resistance to pathogens but determined external damages and reduction of the solubility of cell wall polysaccharides (Lara et al., 2006).

Heated vapour treatment at 52.5 °C or 55 °C during 18-27 minutes of table grape of the ‘Sultanina’ cultivar did not affect weight loss, berry firmness, colour, and total soluble solid and acid content modification. Nevertheless, treatment at higher temperature (58 °C) or for a more prolonged period (55 °C, 30 minutes) reduced fruit quality since it increased weight loss and berry browning (Lydakis & Aked, 2003).

Among effects of heat treatments on tissue senescence of horticultural commodities, it has been shown that the natural yellowish of broccoli is delayed both after exposing it to water dip at 45 °C for 10 minutes or after air conditioning at 50 °C for 2 hours (Funamoto et al., 2003).

Other symptoms of senescence, like geotropism deviation of spears of asparagus, and sprouting of onion, garlic and potato have been controlled by dip treatments in water at 50-55 °C for 2-4 minutes (Cantwell et al., 2003; Lurie, 2006). Pineapple fruit treatment at 38-60 °C for 60 minutes was effective to control internal browning during cold storage (Weerahewa & Adikaram, 2005). Sapote fruit [*Pouteria sapota* (Jacq.) H.E. Moore & Stearn], exposed to disinfestations with dip treatment at 60 °C for 60 minutes showed less pulp browning respect to untreated control (Diaz Perez et al., 2001).

Postharvest treatments of early ripening pears of 'Camusina' and 'Precoce di Fiorano' cultivars with hot dip at 50 or 60 °C for 3 minutes or 1-Methylcyclopropene at 20 °C were useful to delay senescence and internal browning, while soy lecithin and calcium chloride resulted less effective (Mulas et al., 2008a). However, dip treatment at 60 °C determined heat damages in the peel of the two cultivars.

Water dip treatment for 3 minutes a 50 °C and with hot air at 37 °C for 48 hours were effective reducing chilling injury on bloody oranges of the cultivars 'Tarocco', 'Moro', 'Doppio sanguigno', and 'Sanguinello'. Any treatment caused visible damages during quarantine storage of 16 days at 1 °C, subsequent storage of 3 weeks at 8 °C and a further week of simulated shelf life at 20 °C. However, while dip treatment was not influent on fruit firmness, taste, aroma, juice content and composition (total soluble solids, titratable acidity, ascorbic acid and ethanol content), hot air treatment negatively influenced fruit firmness, taste and chemical composition (Schirra et al., 2004).

Postharvest treatments of disinfestations against pests with humid air at 44 or 46 °C, measured inside the fruit during 100 and 50 minutes respectively, do not produce negative effects on 'Olinda' and 'Campbel' oranges (clones of 'Valencia late' cultivar) both for the external appearance and the internal composition (Schirra et al., 2005a). However, these protocols cannot be recommended to the bloody oranges because of the negative influence on fruit quality (off flavours development, fruit softening, high weight loss), and of the reduced resistance to moulds (Mulas et al., 2001).

5.3 Nutritional value

Many studies demonstrated that thermotherapy may influence the biosynthesis of antioxidant or nutraceutical compounds (Schreiner & Huyskens-Keil, 2006) and researches in this direction are increasing. In papaya (*Carica papaya*) fruit, for example, thermotherapy reduces chilling injury, slow down superoxide-dismutase and catalase activities and stops the increase of peroxidase activity (Huajaikaew et al., 2005). Hot air treatment at 34 °C and 50% R.H. during 24 hours of the tomato 'Rhapsody' did not affect to the antioxidant properties of the fruit that developed a normal colour during storage at 10 °C (Soto-Zamora et al., 2005). Otherwise, the fruit exposition to 38 °C during 24 hours in air o in an atmosphere containing a 5% of oxygen determined some negative effects, like loss of antioxidant properties and lack of colour evolution.

Dipping of mango fruit at 50 °C for 60 hours to kill pests may enhance carotene biosynthesis, reduce fruit shelf life, while the thermotherapy associated with cold storage slows carotene development (Talcott et al., 2005). More studies on the cultivar 'Kensington Pride' showed high effectiveness in maintaining fruit quality of thermotherapy in air at 40 °C for 8 hours, or with water dip at 52 °C for 10 minutes (Dang et al., 2008).

Broccoli (*Brassica oleracea* L.) treated at 48 °C for 3 hours with hot air showed slow senescence at 20 °C, better quality and a significant higher contents of chlorophyll, sugars, proteins and antioxidants (Costa et al., 2005). A slow degradation of chlorophyll and an increase in antioxidant properties has been also reported in spinach after water dip treatment at 40 °C for 3 minutes (Gomez et al., 2008).

Dip treatment at 45 °C for 4 minutes of pomegranate fruits (*Punica granatum* L. cultivar 'Mollar de Elche') also produced higher antioxidant activity, total phenols, ascorbic acid, anthocyans, sugars, and organic acids (Mirdehghan et al., 2006).

Studies on different horticultural commodities showed that hot dip at 35 °C for 12 hours (tomato), at 55 °C for 5 minutes (melon), and at 42 °C for 24 hours (mango) inhibited polyphenol-oxidase and peroxidase activities and reduced slow biosynthesis of antocyans, but maintain good nutraceutical properties in the fruits (Cisneros-Zevallos, 2003; Brovelli, 2006; Mulas & Schirra, 2007).

Good results have been obtained with water dip at 50 °C for 2 minutes in order to maintain nutritional and functional properties of kumquat fruit (Schirra et al., 2008d), and the quality of blueberry was also satisfactory after thermotherapy application (Fan et al., 2008).

6. Conclusions and future perspectives

In spite of the beneficial effects, thermotherapy is not sufficient alone to provide protection against postharvest disorders during long term storage (Tab. 1-4). This is because some of the induced mechanisms are only transient. However, it is very clear their synergic effect with the old fungicides and the need of maintaining also this possible supplement of effectiveness for the applications of the new fungicides. In fact there is yet some signal of pathogen resistance for the recently admitted postharvest fungicides.

Further investigations are necessary to optimize protocols for different horticultural commodities, cultivars and zone of production. A good direction to develop more studies is the possibility to combine the thermotherapy with other physical and chemical treatments. In the Tables 1-4 a synthesis of the main effects and indication of the termotherapy is proposed to stimulate new ideas and investigations.

The heat effect on temporary inhibition or enhancement of the enzyme activity, as well as on slowing maturation and senescence of commodities, are some of the evidences more critical to get a general insight of the complex physiological consequences of thermotherapy. The influence of this practice on the biosynthesis of ethylene and of phytochemicals of nutraceutical value, and on the development of aromatic and off volatiles are also some directions for future researches (Cisneros-Zevallos, 2003; Mulas et al., 2006; 2008b; Lafuente et al., 2011).

Commodity	Treatment	Temperature (time)	Effect	References
Apple	Hot humid air	38 °C (4 days)	Mould control. Synergic to antagonist strains and sodium bicarbonate.	Mulas & Schirra, 2007
Apple	Brushing	60-65 °C (15 seconds)	Heat damages	Fallik et al., 2001
Apple	Hot humid air	38 °C (30-120 hours)	Low development of colour and aroma.	Lurie, 2006
Blueberry	Water dip	60 °C (15-30 seconds)	Mould control.	Fan et al., 2008
Broccoli	Water dip. Hot humid air.	45 °C (10 minutes). 50 °C (2 hours)	Delayed yellowish.	Funamoto et al., 2003
Broccoli	Hot humid air.	48 °C (3 hours)	Delayed senescence	Costa et al., 2005
Cactus fruit	Water dip.	52 °C (3 minutes.)	Chilling injury control. Synergic to Thiabendazole.	Schirra et al., 2002b
Cactus fruit	Brushing	65-70 °C (10-30 seconds)	Hot damages.	Dimitris et al., 2005
Citrus (orange, mandarins, lemons, grapefruits, kumquats)	Water dip	50-53 °C (2-3 minutes)	Chilling injury control. Partial control of moulds. Synergic to Thiabendazole and Imazalil.	Schirra & Mulas, 1993; 1994; 1995a; b; c; Schirra et al., 1995; 2002c; 2008c
Citrus	Water dip	50 °C (3 minutes)	Synergic to Azoxystribin, Fludioxonil and Pyirimethanil	Schirra et al., 2005b; 2006; 2010; D'Aquino et al., 2006;
Citrus	Water dip	45-49 °C (150-60 seconds)	Synergic to sodium carbonate and bicarbonate, Thiabendazole and sodium hypochlorite in the mould control.	Palou et al., 2001; Smilanick et al., 2006; Schirra et al., 2008b
Citrus	Water dip Hot humid air	52 °C (3 minutes) 37 °C (48 hours)	Ethanol, acetaldehyde accumulation. Loss of quality.	Mulas et al., 2004; 2006; Schirra et al., 2004
Citrus ('Valencia late' and "bloody oranges")	Hot humid air	44 °C (90 minutes) 46 °C (50 minutes)	Quarantine. Senescence on "bloody oranges".	Mulas et al., 2001; Schirra et al., 2004; 2005a
Citrus ('Tarocco')	Hot humid air.	37 °C (48 hours)	Synergic to Thiabendazole	Schirra et al., 2002a
Citrus (Navelate)	Water dip	53-75 °C (150 seconds)	Hot damages	Palou et al., 2001
Citrus ('Valencia late')	Water dip. Hot humid air.	50 °C (3 minutes) 38 °C (24 hours)	Increased respiration, ethylene production, ethanol and acethaldheyde accumulation	Mulas et al., 2008b
Citrus	Water dip	50 °C (30 seconds)	Synergic to potassium sorbate, Thiabendazole, Imazalil, Fludioxonil and Pyirimethanil	Smilanick et al., 2008

Table 1. Thermotherapy treatments and their different effects.

Commodity	**Treatment**	**Temperature (time)**	**Effect**	**References**
Dates	Hot humid air.	55 °C (2 hours and 30 minutes)	Quarantine.	Rafaeli et al., 2006
Garlic	Water dip	50-55 °C (4-2 minutes)	Avoid spruce	Lurie, 2006
Kiwi	Hot vapour.	47-53 °C (3-6 minutes)	Mould control. Synergic to ethanol	Lagopodi et al., 2009
Kiwi	Hot humid air.	38 °C (30-120 hours)	Low development of colour and aroma.	Lurie, 2006
Lemons	Water dip	49 °C (60 seconds)	Synergic to Thiabendazole	Smilanick et al., 2006
Lemons	Water dip	50 °C (2 minutes)	Synergic to Imazalil	Dore et al., 2009
Loquat	Hot humid air	38 °C (36 hours)	Synergic to antagonist strains.	Liu et al., 2010
Lytchee	Water dip	49 °C (20 minutes)	Heat damages.	Follet & Sanxter, 2003
Lytchee ('McLean's Red')	Water dip	50-60 °C (2 minutes -1 second)	Heat damages.	Sivakumar & Korsten, 2006
Mandarins ('Fortune') and other citrus	Hot humid air.	37 °C (3 days)	Chilling injury control.	Lafuente & Zacarias, 2006
Mandarins ('Dancy')	Hot humid air.	45-48 °C (1-4 hours)	Heat damages.	Lurie, 2006
Mandarins ('Satsumas')	Water dip	45-55 °C (2-5 minutes)	Heat damages. ATPase, pyrophosphatase and antioxidant enzymes.	Ghasemnezhad et al., 2007
Mandarins ('Fortune')	Hot humid air.	37 °C (1-2 days)	Chilling injury control without loss of flavonoids and vitamin C.	Lafuente et al., 2011
Mandarins ('Montenegrina')	Water dip. Brushing.	60 °C (30 seconds)	Synergic to Imazalil	Montero et al., 2010
Mangoes	Hot humid air. Hot vapour. Water dip	51,5 °C (125 minutes) 46-48 °C (3-5 hours) 42-49 °C (7-120 minutes)	Heat damages.	Jacobi et al., 2001
Mangoes	Water dip	50 °C (60 minutes)	Quarantine. Quick maturation.	Talcott et al., 2005
Mangoes ('Kensington Pride')	Water dip. Hot humid air.	52 °C (10 minutes) 40 °C (8 hours)	Mould control.	Dang et al., 2008
Mangoes	Water dip	42 °C (24 hours)	PPO and POD inhibition with antocyans storage.	Mulas & Schirra, 2007
Mangoes	Water dip	50 °C (30 seconds)	Synergic to Fludioxonil and Prochloraz	Swart et al., 2009
Melons	Water dip	55 °C (5 minutes)	PPO and POD inhibition with antocyan storage.	Mulas & Schirra, 2007
Nectarine	Hot humid air.	41-46 °C (24-48 hours)	Heat damages	Lurie, 2006
Nectarine ('Caldesi 2000' and 'Royal Glory')	Water dip	46 °C (25 minutes)	Slow pulp firmness loss.	Malakou & Nanos, 2005
Onions	Water dip	50-55 °C(4-2 minutes)	Avoid sprouting.	Lurie, 2006

Table 2. Thermotherapy treatments and their different effects.

Commodity	Treatment	Temperature (time)	Effect	References
Papaya	Hot humid air.	32,5 °C (10 days)	Heat damages	Lurie, 2006
Papaya ('Sunrise')	Hot humid air.	42 °C (6 hours)	Chilling injury control. Low SOD, CAT and POD action.	Huajaikaew et al., 2005
Peach, plums and nectarines	Water dip.	51.5 °C (1 minutes and 30 seconds.)	Synergic to the fungicide 2,6-D-4-NA	Wells & Harvey, 1970
Peach	Hot humid air.	37 °C (48 hours).	Mould control. Synergic to antagonist strains.	Zhang et al., 2007
Peach	Water dip. Hot humid air.	37-43 °C (1-3 hours.) 37-43 °C (8-24 hours).	Heat damages	Zhou et al., 2002
Peach ('Dixiland')	Hot humid air.	39 °C (44 hours)	Loss of total acidity and pigment increase.	Budde et al., 2006
Peaches, nectarines and apricots	Water dip.	48 °C (2 minutes)	Synergic to Fludioxonil	D'Aquino et al., 2007
Peaches and nectarines	Water dip.	48 °C (6-12 minutes)	Mould control.	Jemric et al., 2011
Pears	Water dip	46 °C (10-20 minutes)	Mould control. Synergic to antagonist strains.	Zhang et al., 2008
Pears ('Precoce di Fiorano', 'Coscia', 'Spadona estiva')	Water dip	50 °C (3 minutes)	Mould control. Synergic to the Fludioxonil	Schirra et al., 2008a
Pears ('Camusina' and 'Precoce di Fiorano')	Wate dip	50 °C (3 minutes)	Partial control of internal bowning	Mulas et al., 2008a
Pineapple	Water dip	38-60 °C (60 minutes)	Chilling injury control	Weerahewa e Adikaram, 2005
Pitaya	Hot humid air.	46,5-48,5 °C (20 minutes)	Quarantine	Hoa et al., 2006
Pomegranate ('Mollar de Elche')	Water dip	45 °C (4 minutes)	Increase of antioxidant activity, sugars and acids	Mirdehghan et al., 2006
Pomegranate ('Wonderful')	Water dip	49 °C (30 seconds)	Synergic to Fludioxonil.	Palou et al., 2007
Pomegranate ('Primosole')	Water dip	50 °C (3 minutes)	Synergic to Fludioxonil.	D'Aquino et al., 2009
Potatoes	Water dip	50-55 °C (4-2 minutes)	Avoid sprouting	Lurie, 2006
Sapote	Water dip	60 °C (60 minutes)	Quarantine. Browning control.	Diaz Perez et al., 2001
Spears of asparagus	Water dip	50-55 °C(4-2 minutes)	Avoid geotropism deviation	Lurie, 2006
Spinach	Water dip	40 °C (3 minutes and 30 seconds)	Delayed senescence and increase of antioxidants.	Gomez et al., 2008

Table 3. Thermotherapy treatments and their different effects.

Strawberry	Water dip	55 °C (30 seconds)	Mould control. Synergic to antagonist strains.	Zhang et al., 2005
Strawberry	Water dip	63 °C (12 seconds)	Heat damages.	Wszelaki e Mitcham, 2003
Strawberry	Hot humid air.	45 °C (3 hours)	Slow pulp firmness loss and colour development. Low acidity.	Vicente et al., 2002;
Strawberry ('Pàjaro')	Hot humid air. Water dip.	40-50 °C (30-75 minutes.) 45 °C (15 minutes).	Heat colour. Low hydrolysis of polysaccharides	Lara et al., 2006
Strawberry ('Feng xiang')	Hit water rinsing and brushing	60 °C (20 seconds)	Mould control.	Jing et al., 2008
Table grape	Water dip	50-60 °C (30-60 seconds)	Mould control. Synergic to ethanol.	Karabulut et al., 2004; Gabler et al., 2005
Table grape	Heated vapour	52,5-58 °C (18-30 minutes)	Heat damages	Lydakis & Aked, 2003
Tomato	Hot humid air.	39 °C (90 minutes)	Delayed colour development.	Ali et al., 2004
Tomato ('Rhapsody')	Hot air with 50% RH	34 °C (24 hours). 38 °C (24 hours.)	Quarantine. Loss of antioxidants and anomalous colour.	Soto-Zamora et al., 2005
Tomato	Water dip	35 °C (12 hours)	PPO and POD inhibition with storage of antocyans.	Mulas & Schirra, 2007
Tomato	Hot humid air.	38 °C (24 hours)	Synergic to antagonist strains.	Zhao et al., 2009; 2010

Table 4. Thermotherapy treatments and their different effects.

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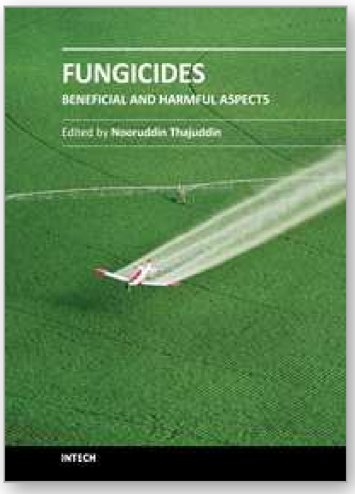

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Fungicides are a class of pesticides used for killing or inhibiting the growth of fungus. They are extensively used in pharmaceutical industry, agriculture, in protection of seed during storage and in preventing the growth of fungi that produce toxins. Hence, fungicides production is constantly increasing as a result of their great importance to agriculture. Some fungicides affect humans and beneficial microorganisms including insects, birds and fish thus public concern about their effects is increasing day by day. In order to enrich the knowledge on beneficial and adverse effects of fungicides this book encompasses various aspects of the fungicides including fungicide resistance, mode of action, management fungal pathogens and defense mechanisms, ill effects of fungicides interfering the endocrine system, combined application of various fungicides and the need of GRAS (generally recognized as safe) fungicides. This volume will be useful source of information on fungicides for post graduate students, researchers, agriculturists, environmentalists and decision makers.

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