Innovations in the Control of Postharvest Diseases of Fresh Fruits and Vegetables

S. M. Sanzani[1], F. Nigro[1], M. Mari[2] and A. Ippolito[1]
(1) Dipartimento di Protezione delle Piante e Microbiologia Applicata, University of Bari, Via Amendola 165/A, 70126 Bari, Italy; (2) CRIOF, Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, Via Fanin 46, 40127 Bologna, Italy, Email: ippolito@agr.uniba.it

Abstract

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The establishment of Euro-Mediterranean Free Trade Area (EMFTA) in 2010 is expected to accelerate trade growth in different fields, including the market in fresh fruit and vegetables from south, south-east Mediterranean countries to foreign countries. In this context, effective cold chain and management practices of fresh produce are required, also considering that safety is an additional prerequisite for market entry. Traditionally, chemical fungicides have been used effectively by exporters to ensure high quality of fruits and vegetables over extended periods of storage or transportation. However, the increased global chemophobia and the reduced efficacy of chemicals due to pathogen resistant strains, have forced producers to evaluate more safe alternatives for controlling postharvest diseases in a context of sustainable agriculture. Several means, such as natural compounds of animal and plant origin, organic and inorganic salts, antagonistic microorganisms, elicitors to induce natural host defenses, physical means as ultraviolet illumination, hypobaric pressure, hot water, modified atmosphere and packaging, and integrated control strategies, represent some of the approaches recently evaluated, and to some extent already applied, to ensure top fruit quality. This review deals with the substantial progress obtained by researchers in the use of alterative control means, also taking into account constraints and obstacles still making difficult their large diffusion and practical application.

Keywords: Postharvest diseases, natural products, antagonistic microorganisms, Mediterranean countries

Introduction

In recent decades agriculture has undergone deep changes in order to adapt itself to the fast evolution of the market and to the changed requirements of the consumers. The concentration of specific products in specific areas and at certain times of the year determines dangerous phenomena of overproduction, unprofitable prices and difficulty in placing products on the market. On the other hand, transport by road, rail, sea or air allows fruit and vegetables to reach large areas of the world. However, to be distributed over time and space, these products need to be subjected to a short or long retention period, in relation to product characteristics and market demands. For example, strawberries are stored for a few days (2-3), while pear, apple, and kiwifruit endure longer retention periods (up to 7 months), during which they tend to increase their organoleptic characteristics.

Since rich in water and nutrients, during storage fruits are an ideal substrate for the development of pathogenic microorganisms, establishing processes of rot with consequent losses of product ranging from a minimum of 10-15% in countries with advanced technologies, to over 50% in developing countries (34). Since the beginning of the 60s, the use of fungicides and storage technologies (controlled atmosphere, low oxygen atmosphere, etc.) have extended fruit postharvest life, significantly reducing their losses. However, fungicide use is strictly regulated by the EU and national legislation of EU Member States, and currently very few active ingredients are allowed against postharvest diseases; for some products, such as stone fruit and strawberry, no postharvest fungicide treatments are allowed. The described situation, the need for an agriculture that meets the new challenges posed by EU enlargement, the increasing consumer attention towards fruit and vegetables free from pesticide residues, the legislative restrictions aiming at increasing food security, the development of pathogen strains resistant to the few admitted postharvest fungicides and the need for high-quality products, have increased the search for defence systems alternative to chemicals. This review deals with the substantial progress obtained in the use of alterative control measures, also taking into account constraints and obstacles, still making difficult their large diffusion and practical application.

Alternative control measures

Alternative control measures developed over more than 20 years of research include use of: 1) antagonistic microrganisms, 2) compounds of natural origin, 3) food additives, 4) physical measures.

Antagonistic microorganisms

Several commercial products have been registered, such as the biofungicides BioSave® 100 and 110 (JET Harvest Solutions, Longwood, PL) based on two strains of *Pseudomonas syringae*, used to control pome fruit, citrus, cherry, and potato diseases, and Shemer® (AgroGreen, Asgdod), based on a strain of *Metschnikovia fructicola*, marketed in Israel against postharvest diseases of sweet potatoes, table grapes, strawberries, peppers, and carrots. In Canada, the Neovo Technologies (Abbotsford, British Columbia) is developing a product based on *Candida saitoana*. In Europe, the registration of CandiFruit (Sipcam Inagri, SA Valencia), based on *Candida sake*, has been recently obtained, although limited to Spain, and in Spring 2010 a product based on *Pantoea agglomerans* (Pantovital, Domco, Spain) is expected to be commercialized; in Belgium, BioNext (Gembloux) is developing a product based on *Candida oleophila*. However, biological control agents are not yet part of the regular postharvest practices and the reasons are basically three: 1) inadequate and inconsistent antifungal activity, 2) difficulty in obtaining a suitable formulation, and 3) uselessness in controlling pathogens causing latent or quiescent infections. Results from semi-commercial scale trials showed that antagonists were not able to reduce infections of citrus and apples at a practical acceptable levels. Furthermore, a different level of efficacy was observed when the same antagonist was applied to fruit from different orchards or with different degree of maturity. This may be ascribed to the quality of the fruit, the inoculum density of the pathogen in the orchard, the level of host susceptibility, the time elapsing between the infection and the treatment, and the possible presence of latent or quiescent infections.

The formulation plays an important role not only on the effectiveness of the antagonist, but also on its applicability and costs. The development of a formulation involves the identification of a substrate that allows for maximum production of antagonist cells at a limited cost. Subsequently, the stability of the formulation has to be guaranteed over the time, with an activity comparable to that of fresh cells. For postharvest pathogens, such as *Penicillium expansum* and *P. digitatum*, the establishment of the infection process requires the presence of a wound and an environment rich in nutrients. This environment is also suitable to the antagonist, that can immediately grow and colonize the wound, thus preventing pathogen germination and colonization. Whereas concerning *Botrytis cinerea*, *Monilinia* spp., and *Phlyctema vagabunda* that cause latent infections, their control is very difficult because the antagonist is not able to reach internal colonized tissues. To overcome these drawbacks, the integration of antagonists with other alternative control measures has been suggested, considering the possible additive or synergistic effect. To this regard, different combinations have been studied: with food additives, air and hot water, resistance elicitors, etc. Food additives are essentially fungistatic, delaying spore germination and thus the whole infectious process. A practical example of integration of an antagonist with resistance elicitors is given by two products marketed by Neovo Technologies, made up of cells of *C. saitoana* in combination with chitosan (InnovaCoat) or lysozyme (InnovaCure). In case of formulations containing the antagonist alone, the control of latent infection would be possible with the use of a strategy involving a preharvest application (15). Formulations as Shemer® and Serenade® (AgraQuest, based on *Bacillus subtilis* and registered in the US for preharvest use), applied at critical stages for the establishment of latent infections in the field (flowering, fruit growth) gave satisfactory results in controlling storage rot on strawberries, grapes and citrus (3, 18).

Compounds of natural origins

Plants have a large set of secondary metabolites associated with the defence system, many of which also function as inhibitors of fungal pathogens (10). These compounds are generally concentrated in the outer cell layers and can be constitutive or inducible, i.e. produced as a result of biotic or abiotic stresses. Their antifungal activity is considered extremely attractive because of the scarce toxicity at low concentrations. Moreover, the high volatility makes some of these compounds particularly suitable for gaseous application. However, volatile compounds normally have a strong smell and can be absorbed and metabolized by fresh fruit, thus altering their taste and, when used at high concentrations, can be phytotoxic to plant tissues. Several aqueous or ethanol extracts of various plants, even spontaneous (6, 8), were evaluated *in vitro* and *in vivo* against a range of postharvest pathogens. Generally, in these assays only a small percentage of extracts show significant antifungal activity. Moreover, the presence of active compounds in the extracts is not stable over time and it is usually highest in extreme environmental conditions. The plant organ from which extraction takes place can affect their composition: extracts from stems are often more effective than those from leaves and flowers. This high variability in composition and concentration of active substances makes difficult to obtain a standard product for possible formulation. Therefore, it seems desirable to purify plant extracts, even partially, so to obtain standardized compounds to be used at postharvest phase (31). Among the antimicrobial substances occurring in plants, the isothiocyanates show interesting prospects for postharvest application. This group comprises more than 130 different compounds, mainly distributed in the family of *Cruciferae* (cauliflower, Brussels sprouts, etc.). Their hydrolytic products proved to be toxic to microrganisms and seem to play an important role in plant resistance to disease (5). Some of these compounds are volatiles that could be successfully applied to fruits and vegetables as vapour treatments before storage by a process called 'biofumigation'. Moreover, pharmacological studies reported that isothiocyanates produce beneficial effects on human health (33). Another substance particularly studied is *trans*-2-hexenal, an aromatic compound found in many plant products such as tea, olive oil, and fruit. Its production increases rapidly when plant tissues are damaged or injured by biotic and abiotic factors and can play an interesting role in controlling the expression level of defence genes (7). The compound has shown some interesting fungicidal activity against several pathogens including *Alternaria alternata* and *B. cinerea* (13). In particular, a *trans*-2-hexenal treatment of Golden Delicious apples 24 hours after inoculation significantly reduced not only the percentage of fruit infected by *P. expansum* but also their patulin content, without altering the organoleptic fruit qualities (23). In contrast, treatment with *trans*-2-hexenal on apricots, nectarines, peaches, and strawberries, while showing a good antifungal activity caused phytotoxic effects (23). Therefore, the evaluation of organoleptic characteristics of processed fruits is essential to identify a control strategy applicable at a practical level. Finally, in this category, compounds of microbial or animal origin, extracted or released, can be included. An interesting study was performed on molecules produced by an endophytic fungus, *Muscodor albus*, with antifungal activity against a wide range of pathogens (22). The fungus produces at least 28 volatile compounds whose mixture can be more effective than its individual components. The application of *M. albus* as biofumigant is still under study, since it has some drawbacks, such as poor efficacy when grown at low temperatures (3°C). A possible solution could be growing

M. albus in an heated environment outside the cold room and subsequently conveying its volatiles within the room (32). Other examples of metabolites of microbial origin are represented by iturin, an antibiotic produced by several strains of *B. subtilis* effective against brown rot of stone fruit (12), and pyrrolnitrin, synthesized from strains of *Pseudomonas cepacia*, active against *B. cinerea* on raspberry and strawberry, and against *P. expansum* on apples and pears (9). Despite the possible arising of bacterial strains resistant to antibiotics discourages its use, pyrrolnitrin has been used as a template for the synthesis of new fungicides with low environmental impact. Among the substances of animal origin, chitosan, a biodegradable polymer produced commercially from the chitin (abundant in the esoskeleton of arthropods and in the wall of certain fungi), has been widely tested against postharvest diseases of fresh fruit and vegetables. Chitosan is used in various fields, e.g. as fining agent for wines and beers, moisturizer in cosmetics, drug in diets and, more recently, in agriculture. For its ability to form a thin film, chitosan acts as a barrier to the diffusion of gases reducing fruit and vegetables weight loss and delaying senescence. Moreover, when applied at pre- or postharvest stage, the polymer is able to reduce rots on table grapes, mangoes, cherries, papaya, citrus, etc. (14, 29). The activity of the polymer depends on the acid in which is dissolved (acetic acid is usually preferred) and the substances with which complexes (e.g. zinc significantly improves its direct antimicrobial activity). Several interesting studies on the preharvest application of this polymer report its ability to delay the reactivation of latent and quiescent infections in the mature fruit, resulting in a longer shelf-life (15). Various formulations used against root and leaf diseases are on the market, but the only proposed postharvest formulation is "InnovaCoat" from Neovo Technologies, which contains the compound in combination with an antagonist.

Food additives with antifungal activity

Salts such as sodium and potassium carbonate and bicarbonate, calcium chloride, potassium sorbate, calcium propionate, etc., also called “Generally Recognized as Safe” (GRAS) substances, have aroused some interest as possible alternative control measures against postharvest pathogens. They have peculiar characteristics including low toxicity, high solubility, and relatively low costs. They are also commonly used to preserve many foods (cheese, vegetable products, sauces, and meat) from microbial spoilage and are able to inhibit the production of mycotoxins by fungi such as *Aspergillus flavus*, *A. parasiticus* (4), *P. expansum*, and *P. patulum* (19). There are numerous studies on the use of sodium and potassium carbonate and bicarbonate and calcium chloride on various fruit and vegetables (27), alone or in combination with other alternative control measures (16). Although they are not yet registered as pesticides, their proven effectiveness would encourage a practical application in various production systems. In this regard, potassium bicarbonate has been recently added to the list of substances available in organic farming, but is not registered for postharvest use; when applied just prior harvest it resulted effective in controlling green and blue mould on citrus and gray mould on table grapes (26, 36). Similarly, sodium bicarbonate significantly reduced infection by *P. expansum* on apples (17), whereas potassium sorbate showed good activity against *Monilinia* spp. on peaches and nectarines (11).

Physical measures

They include thermotherapy, curing, UV light, treatments at pressures higher or lower than the atmospheric one, ozone, etc. Physical stress may have a dual effect on the fruit: it is active against the pathogen on the fruit surface and in the environment and at the same time induces host defence response (35). The main advantages of physical measures can be summarized as follows: 1) simple to implement; 2) able to inhibit the germination of fungal spores on the fruit surface; 3) relatively inexpensive; 4) no residues, therefore healthy for human and environment. Heat can inhibit the pathogen localized in the inner layers of the epicarp (2) or within the lenticels, as in the case of *Phlyctema vagabunda* (24). Moreover, it not only reduces the rate of infections, but seems to have a beneficial effect on fruit resistance to cold damage. A reduction of some abiotic stresses related to cold storage was recorded in several fruits including plums (1) and apple cv 'Pink Lady' (Mari, unpublished data). On the other hand, the physiological response of the fruit may differ depending on the variety, season and location of the crop, so it is essential to establish a proper time and temperature of treatment. Among the physical measures, the UV light of short wavelength (UV-C, 280-100 nm) shows interesting perspectives of applications due to the direct activity against pathogens (germicidal activity) and the resistance induction in the host (hormesis). The germicidal activity, however, is evident only at high dosage that results in damage to fruit tissues (21). Hormetic doses of UV-C light have been shown to induce production of antifungal compounds, ripening delay, and reduction of chilling injury. Optimal doses for reduction of rots are usually low (0.25-5 KJ/m^2), but vary depending on the species and vegetables: e.g. on grape and strawberry levels of 0.125-0.5 KJ/m^2 and 0.5-1 KJ/m^2 were found to be effective against gray mould (25, 26), while higher doses were necessary to contain peach rots caused by *M. fructicola* and apple rots caused by *Alternaria* spp. and *Monilinia* spp. (20). Furthermore, the UV-C light at low doses seems to support the population growth of antagonistic microrganisms (bacteria, yeasts and yeast-like fungi) on the fruit surface (25). Although in many cases UV-C has shown the ability to reduce disease development, its low efficiency doesn’t allow a practical application, except in case of integrated approach. Among the physical measures, even hypobaric and hyperbaric treatment might be considered a promising alternative to synthetic fungicides. Preliminary tests have determined significant rot reductions on sweet cherries and table grapes (30). Hypobaric treatments have been found effective in containing the postharvest rot of strawberries, sweet cherries and table grapes, especially when applied in combination with chitosan (28). The treatments at pressures other than the atmospheric one would offer the great advantage that the fruits are not treated with any type of product (microorganism, natural substance, GRAS, etc.), that no matter how innocuous, always raise consumer concerns. However, to date, equipment costs and scarce operational effectiveness make them far from practical use.

Conclusions

Over the last two decades the increasing interest towards control measures alternative to synthetic fungicides against postharvest pathogens has produced numerous studies with encouraging results. On the other hand, almost all the measures described above, taken individually, are unable to obtain an economically acceptable level of control, so numerous studies are in progress to improve their effectiveness. In the case of biocontrol agents, the formulation is still a key issue, together with the compatibility with the normal practice of fruit processing and storage. As already mentioned, it is also important the integration of biocontrol agents with GRAS, hot water, or resistance elicitors, in order to exploit additive or synergistic effects between different control strategies. Moreover, research has highlighted some critical points that can be summarized as follows: i) difficulty in the registration of biofungicides; ii) potential toxicity to humans and the environment of so-called natural molecules and their possible phytotoxicity when used at high concentrations; iii) need for a exhaustive study on the environmental fate and dispersal of the antagonist. Moreover, the development of new fungicides based on natural compounds should include detailed investigations on their degradation in foods or in biological systems, although their natural origin makes them relatively biodegradable and with negligible residues.

الملخص

سانزاني، سيمونا ماريانا، فرانكو نجرو، م. ماري وأنتونيو إيبوليتو. 2009. المستجدات في إدارة أمراض ما بعد الحصاد للفواكه والخضار الطازجة. مجلة وقاية النبات العربية، 27: 240-244.

سيسهم إنشاء منطقة تجارية حرة أوروبية- متوسطية في عام 2010 في تقوية النشاطات التجارية في مجالات مختلفة بما فيها الخضار والفاكهة الطازجة بين أسواق جنوب وجنوب شرق المتوسط والأسواق الخارجية. وفي هذا السياق، يعد وجود سلسلة للتخزين المبرد والإدارة الجيدة للمنتجات الطازجة من مستلزمات النجاح في هذا المجال. كما أو وجود الإنتاج الآمن هو ضرورة ملحة لدخول هذه الأسواق. تستعمل المبيدات الفطرية عادة للحفاظ على نوعية جيدة من الخضار والفواكه خلال فترة التخزين والنقل. إلا أن الخوف العالمي من استخدام المركبات الكيماوية بالإضافة إلى تطور سلالات جديدة من الممرضات مقاومة لهذه المبيدات، دفع المنتجين لاستخدام بدائل أكثر أماناً لمكافحة أمراض ما بعد الحصاد. إن استخدام مركبات طبيعية من أصل نباتي أو حيواني، أملاح عضوية أو غير عضوية، كائنات دقيقة ذات قدرة تنافسية، مركبات تزيد من مقاومة النسيج النباتي، طرائق فيزيائية مثل الأشعة فوق بنفسجية، ماء ساخن جو تخزين محوّر وطرائق متكاملة تشكل الأساس لعدة طرائق تم تقييمها حديثاً، منها ما هو حالياً قيد الاستعمال. تعدّ هذه الورقة مراجعة علمية للتقدم الذي أحرزه الباحثون في استخدام الطرائق البديلة، كما سيتم مناقشة العقبات التي تعترض سبيل استخدام بعض الطرائق بشكل واسع.

كلمات مفتاحية: أمراض ما بعد الحصاد، مركبات طبيعية، بلدان البحر المتوسط، أحياء دقيقة تضاددية.

عنوان المراسلة: أنتونيو إيبوليتو، قسم وقاية النبات والميكروبيولوجيا، جامعة باري، 70126 باري، ايطاليا، البريد الإلكتروني: ippolito@agr.uniba.it

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