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State of the Art and Challenges of Post-harvest Disease Management in Apples

M.H. Jijakli and P. Lepoivre

ABSTRACT: Despite modern storage facilities, losses from 5 to 25% of apples are still being recorded in storage room. Fungal pathogens such as *Botrytis cinerea*, *Penicillium expansum* and *Gloeosporides* group are mainly responsible of important economical losses even if physiological disorders (bitter pit, water core and storage scald) cannot be neglected. Post-harvest disease control is a complex problem which cannot be solved by a single solution. The control of factors affecting the fruit physiology with pre- and post-harvest handling practices, the sanitation and the application of synthetic fungicides in pre- and post-harvest treatments are the primary means of controlling post-harvest diseases. However, the future use of fungicides is uncertain due to the development of pathogen resistance, the consumer reluctance to chemical residues in food and environment and the consequent growing scarcity of fungicides aimed at post-harvest situations. Several novel approaches (including biological control agents, natural biocides and induction of fruit defence mechanisms) are emerging as possible alternatives to synthetic fungicides. However, the complete replacement of the chemical pesticides by one of these alternative methods is unrealistic because of their lack of efficacy in case of high disease pressure. These alternative methods must be integrated in association with limited quantities of fungicides, as well as efficient management and handling practices to combat diseases in harvested apples. This novel IPM approach should be completed by further studies on predictive models of post-harvest disease development and genetic resistance.

1. Introduction

Apple trees belong to the family of Rosaceae. These fruit trees are grouped under the name *Malus* x *domestica* Borkh. (Bondoux, 1992). However, the origins of the actual cultivated varieties are complex and remain uncertain (Jones and Aldwinckle, 1990). In 2000, the apple production reached around 60 millions tons in the world (FAOSTAT, 2002). The same year, Western European apples production (European

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Union 15 countries + Switzerland + Norway) was the first worldwide producer with 9.64 millions tons of harvested fruits whilst Eastern European production was of about 3.50 millions tons. USA constitutes another major producer of apples (4.8 millions tons). Brazil, Argentine, Chile, South Africa, New Zeeland and Australia are also important apple growers with 1.16, 0.83, 0.75, 0.65, 0.48 and 0.33 millions tons of fruits, respectively.

The surface extension of orchards since 1950 was partially due to the improving of storage methods. These methods allowed to extend the life period of harvested apples and to spread out their commercialization. Despite these modern storage facilities, post-harvest diseases of apple annually cause losses of 5-25 %, since early 1970s (Bondoux, 1992). Accurate data on the scale of losses are difficult to obtain and, where fungicidal treatments are applied either before or after harvest, only indicate the incidence of those species which survive such treatment. The most accurate surveys were made in the 1960s (Edney, 1983). Nevertheless, fungal pathogens are responsible of important economical losses even if physiological disorders cannot be neglected.

Until now, post-harvest diseases of apples are largely controlled by pre- and post-harvest handling practices and the application of synthetic fungicides. However, the possible deregistration of effective and widely used fungicides (Wellings, 1996), the development of fungicide-resistant strains of post-harvest pathogens (Franclet, 1994) and the increase of Integrated Pest Management (IPM) (or Integrated Fruit Production, IFP) and organic culture in the context of sustainable agriculture (Cross, 2000) increased the demand to develop alternative methods to control diseases. That need is strengthend by consumer reluctance to chemical residues in food and public concern for environmental safety. Several novel approaches are emerging as alternatives to synthetic fungicides. The purpose of this work is to describe principal post-harvest diseases and present conventional and emerging methods for controlling post-harvest diseases of apples.

2. Fungal diseases

The importance of each fungal pathogen can vary from one country to another. In Belgium and France (Bondoux, 1992) most losses are

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attributable to *Penicillium expansum* Link, *Botrytis cinerea* Pers., and the Gloeosporides group. In USA and UK, *B. cinerea* and *P. expansum*, *Pezicula malicorticis* (H. Jacks.) Nannfs. and *Mucor piriformis* E. Fischer are the most important agents of post harvest diseases (Rosenberger, 1991). For practical reason, the classification of fruit diseases due to fungal pathogens is based on their mode of penetration in the fruit and their further evolution (Table 1).

TABLE 1
Characterisation of common postharvest fungal diseases

	Disease name	**Causal agent**	**Source of contamination**	**Incidence**
Lenticel rot[1]	Bitter rot	*Colletotrichum gloeosporioides*[2]	Cankers	Economical incidence worldwide
	Bull's eye rot	*Cryptosporiopsis curvispora*[2]	Cankers	Economical incidence particularly in Pacific Northwest and in Europe
	Gloeosporium rot	*Trichoseptoria fructigena*[2]	Cankers and leaves	Economical incidence particularly in Europe
Core rot[1]	Mouldy core rot and dry core rot	*Alternaria* spp.	Dry organs	Local and/or sporadic incidence
Eye rot[1]	Dry eye rot	*Botrytis cinerea*[2]	Various debris	Local incidence in North America, Europe and New Zealand
Wound pathogens	Blue mould	*Penicillium* spp	Various debris	Economical incidence worldwide
	Grey mould	*Botrytis cinerea*[2]	Various debris and cankers	Economical incidence worldwide
	Brown rot	*Monilinia fructicola*[2] *and M. fructigena*[2]	Mummified fruit and cankers	Economical incidence in North America, New Zealand and Australia (M *fructicola*) and in Europe (*M. fructigena*)
Other alterations	Mucor rot	*Mucor piriformis*	Organic matter	Economical incidence in USA
	Phytophtora rot	*Phytophthora syringae* and *P. cactorum*[2]	Soil and cankers	Sporadic incidence in Europe

1: latent infections, 2: species able to infect other organs than fruits

2.1. Latent infection

Post-harvest decay attributable to latent pathogens results from infections that occur in the field but remain quiescent and escape notice at harvest.

2.1.1. Lenticel rot

Various fungal species are able to infect fruits through lenticels. These species belong to the "Gloeosporides" group and constitute one of the major source of post-harvest apple losses.

"Bitter" rot is caused by *Colletotrichum gloeosporioides* (Penz.) Sacc. (syn. *Gloeosporium fructigenum* Berk.). The teleomorph is *Glomerella cingulata* Stoneman. Bitter rot is a common disease of apples in practically all countries where they are commercially grown (Fig. 1). Lesions originating from infections by conidial types (which produce only conidia) are circular and become sunken when they enlarge (Jones and Aldwinckle, 1990). Acervuli are produced in concentric circles around the infection point. Lesions initiated by perithecial types (which produce both ascospores and conidia) are darker brown and not sunken. Conidial masses associated with these perithecial types are first orange-salmon in early stage and turn to dark brown. Fruit infection can occur before, during or just after bloom. Fruit are equally susceptible during all stages of development (Bondoux, 1992). At the optimum temperature (26°C), latent infection can occur with a wet period as short as 5 hours (Koelher, 2000).

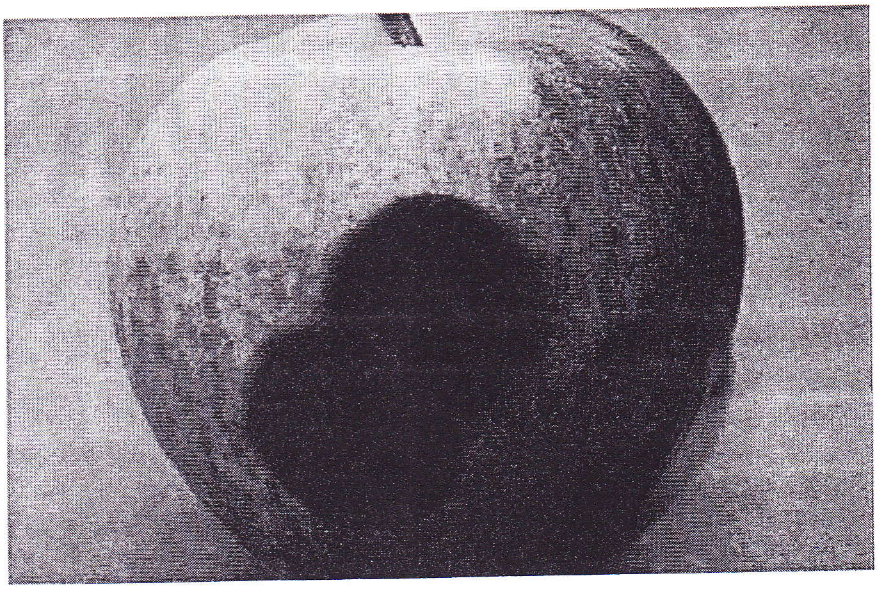

Fig. 1: Biter rot (provided by P. Creemers, Royal Research Station of Gorsem, Belgium)

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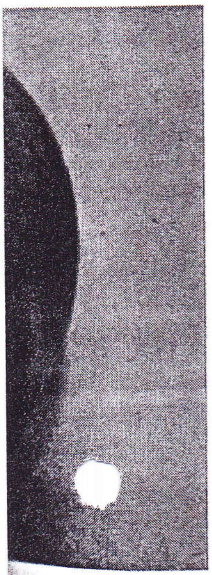

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The causal agent of "bull's eye rot" is *Cryptosporiopsis curvispora* (Peck) Gremmen (syn. *Gloeosporium perennans* Zell. et Childs). The perfect stage is *Pezicula malicorticis* [syn. *Cryptosporiopsis malicorticis* (Cordl.) Nannf.]. Bull's eye rot seems to be an important disease in the Pacific Northwest and in Europe. It is more effective parasite of woody tissue and was initially described as a "perennial canker" (Edney, 1983). *C. curvispora* causes circular spots around the lenticels which grow slowly. Lesions appear slightly sunken and brown with a lighter brown centre. Numerous lesions can occur on the same fruit and are originated from different lenticels (Creemers, 1998). Conidia are produced in acervuli throughout the year and dispersed by rain. Fruit can become infected anytime between petal fall and harvest. Fruit susceptibility increases as the growing season progresses (Jones and Aldwinckle, 1990).

"Gloeosporium rot" is due to *Trichoseptoria fructigena* Maubl. [syn. *Gloeosporium album* Osterwalder, teleomorph *Pezicula alba* (Grunthr.)]. This species is an important parasite of dessert apples, particularly in Europe. *T. fructigena* is very similar to *C. curvispora* in terms of symptoms and biology. Nevertheless, it parasitises wood with difficulty and is found mainly on dead wood (Edney, 1983). As the conidia are produced throughout the year, fruit may become contaminated at any time during the growing season.

Other species can provoke occasionally and/or locally some losses may be due to fungi such as *Cylindrocarpon mali* (All.) Wr., *Alternaria* spp., *Stemphylium botryosum* Wallr. and *Cladosporium herbarum* Lk. (Bondoux, 1992).

2.1.2. Core rot

Most cultivars susceptible to core rot (*i.e.* 'Gloster', 'Belle de Boskoop'…) have an open sinus extending from the calyx into the core (Creemers, 1998). Mouldy core may develop into dry core rot if the pathogen penetrates into the core flesh. But the fungus is generally limited to the core or carpel region (Jones and Aldwinckle, 1990). External symptoms are rare, except infected fruit may colour and fall prematurely. Several fungi [such as *Alternaria* spp., *Stemphylium* spp., *Cladosporium* spp., *Phomopsis mali*, *Fusarium avenaceum* (Fr.) Sacc., *Trichothecium roseum* (Bull.) Lk.] can be associated with mouldy core and dry core rot (Bondoux, 1992 ; Creemers, 1998; Jones and Aldwinckle, 1990).

2.1.3. Eye rot and calyx end rot

Dry eye rot or blossom-end rot has been reported on apples in North America, Europe and New Zealand (Jones and Aldwinckle, 1990). The first symptoms (red discoloration) appear at the base of one or more of the sepals on the calyx end of the fruit. Dry-eye rot evolves as a shallow, hard rot over a small area often with a red border. These alterations are often due to *Botrytis cinerea* but other species (*Cylindrocarpon mali* and *Alternaria* spp.) can also provoke similar symptoms.

Nectria galligena Bres. is the causal agent of "Nectria canker" that can kill young trees and branches of older trees, can also infect apple fruit resulting in an eye rot disease. This eye rot is characterised by slightly depressed, brown necrotic areas on the fruit surface (Jones and Aldwinckle, 1990).

Calyx end rot is a sporadic and minor disease of apple fruit which is characterised by a soft rot. It may expand to cover about 1/3 of the end of a fruit.

It is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary (Jones and Aldwinckle, 1990 and Koelher, 2000).

2.2. Wound pathogens

All the post-harvest pathogens on apples are potential wound pathogens. In practical conditions, only some fungi which are able to fast growing are responsible for important apple loss (Bondoux, 1992). When fruit has been weakened by ripening and aging, the infection speed is comparable to growth on an artificial medium (Creemers, 1998). These fungal agents infect fruits by deposition of airborne or waterborne conidia on wounds during harvesting, transport and handling before storage (Jijakli *et al.*, 1999). The higher frequency of apple wounds with the mechanisation of the harvest and conditioning processes before storage explains the increased importance of wounds pathogens.

At least 11 species have been isolated from naturally infected pome fruits exhibiting "blue mould" symptoms, the most frequent being *P. expansum*, *P. solitum* Westling and *P. commune* Thom (Jones and Aldwinckle, 1990). The species that causes the most extensive decay is *P. expansum* (Fig. 2). Most infection caused by *Penicillium sp.* is

Fig. 2: Blue mould (provided by P. Creemers Royal Research Station of Gorsem, Belgium)

initiated at wound sites, such as cuts and stem punctures, but fruit can also become infected through lenticels on unbroken skin, particularly at bruise sites. Typical symptoms of blue mould are circular, tan coloured lesions with sharp margins between the watery soft rot and healthy fruit flesh (Bondoux, 1992). Production of blue-green spores can occur on the surface of the decay. They form a dense, powdery mass at the centre of the lesion (Jones and Aldwinckle, 1990). *Penicillium* spp. can be isolated from orchard soils, but the disease is rare in the field except on fruit that have fallen to the ground. Airborne conidia originating from decayed fruit or from sporulation on bins and storage walls are present in packinghouses and storages.

"Grey Mould" (*Botrytis cinerea*) is also a common worldwide decay of apples (Fig. 3). Grey mould lesions are characterised by pale tan mouldy areas without sharp margins, older portions of the decay turning darker brown (Bondoux, 1992). Darker spots around the lenticels may appear on some varieties. Sporulating mycelium is gray, but little sporulation occurs at cold-storage temperatures (Jijakli and Lepoivre, 1998). Black sclerotia can be produced on fruit, especially around the infection site. The source of *Botrytis* spores is the orchard. The fungus grows and sporulates abundantly on dead and dying plant

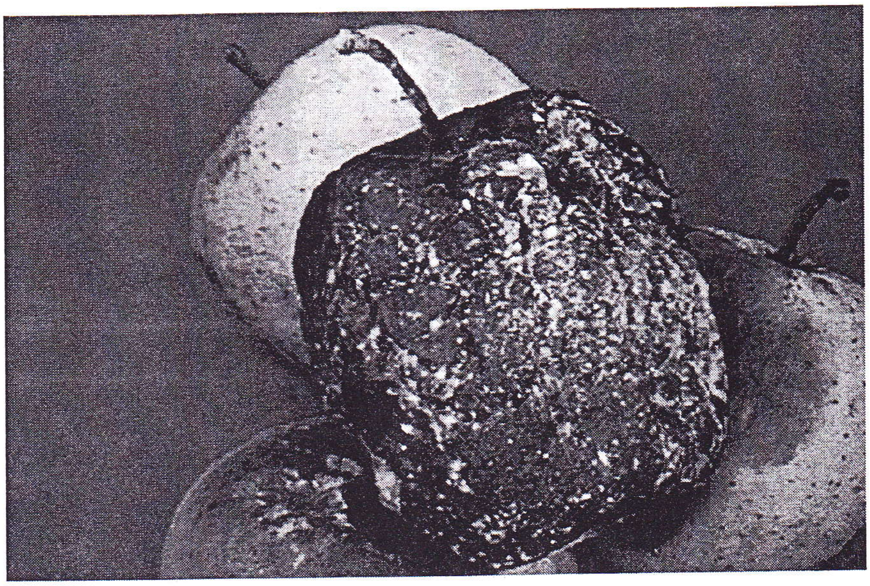

Fig. 3: Grey mould (provided by P. Creemers, Royal Research Station of Gorsem, Belgium)

material found in orchard cover crops, especially during cool, moist weather (Jones and Aldwinckle, 1990). These initially rotted fruits spread the disease through fruit contact to produce nests of decaying fruit.

Three species of *Monilia* cause "brown rot" of apple (Fig. 4). *M. fructicola* (Wint.) Honey is established throughout North America, New Zealand and Australia and attacks injured apples as they ripen in some occasions (*i.e.* a buildup of the disease on neighbouring stone fruit crops). This pathogen has recently been isolated in France from stone fruits but not yet from pome fruits (Lichou *et al.*, 2002). *M. fructigena* Pers. is the most common species in Europe and is considered as a major disease in that geographical area (Jones and Aldwinckle, 1990). The third species, *M. laxa* (Ehrenb.) Sacc., is rare on apple on which it can cause fruit rot. Superficial, circular brown spots expand outward on the surface of the fruit and result in a soft decay of the flesh. Spots of gray-white fungus may develop on the surface of the lesions and are arranged in concentric band (Jones and Aldwinckle, 1990). *M. fructigena* overwinters in infected peduncles or twig cankers on branches and produces conidia which are disseminated by rain and infect blossoms. Conidia, produced on infected blossom and twigs, infect wounded fruit as they mature.

Fig. 4: Brown rot (provided by P. Creemers Royal Research Station of Gorsem, Belgium)

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2.3. Other post-harvest fungal diseases

"Mucor rot", caused primarily by *Mucor piriformis*, is less frequently encountered than *B. cinerea* and *Penicillium spp.*, but can be highly destructive (Sanderson, 2000). Losses due to this disease have been serious in United States. Lesions are watery with less distinct margins than those in fruit with blue mould (Jones and Aldwinckle, 1990). Fruit are relatively quickly decayed with the entire fruit often involved so that little is left of the fruit a few months after infection occurs. Similar to gray mould, mycelia are often present on the surface of diseased fruit. They are water and insect disseminated and can contaminate packinghouse water systems.

"Coprinus rot" (*Coprinus psychromorbidus* Redhead and Traquair) has been found throughout the Pacific Northwest. It is often mistaken for bull's eye rot. Fruit infection occurs during the last month before harvest. This fungus appears as a white, cobwebby growth on the surface of infected fruit and will create nest or cluster rot like gray mould (Kupferman, 1993).

"Alternaria rot" [*Alternaria alternata* (Fr.) Keissler] may occur on apples in any production stage (Kupferman, 1993). This fungus lives on dead and decaying plant tissue in the orchard. Spores contaminate fruits in the orchard and during the handling process. The amount of decay depends on the condition of the fruit. Infection usually occurs through breaks in the skin or other weakened areas caused by sunburn, bruising, chemical injury or scald.

"Phytophtora rot" is caused by *Phytophthora syringae* (Klebahn) Klebahn and *P. cactorum* (Lebert and Cohn) Schröter. It is usually of sporadic occurrence and of limited economical importance (Creemers, 1998). Nevertheless, this disease provoked important economical losses in several European countries in the 1970's due to high humid conditions. Rotted fruits are typically marbled olive green or brown to uniformly pale brown in apple. Rotted flesh has a alcoholic odour and vascular tissue are dark-stained. Both pathogens perennate as oospores in apple orchard soils. Fruits rot epidemics are associated with high rainfall in cool weather for *P. syringae* and in warm weather for *P. cactorum*. Inoculum is splashed onto fruit and infection occurs *via* the lenticels.

3. Physiological disorders

A number of disorders in fruit are known as physiological because they are not the result of damage by micro-organisms or insects. The physiological disorders (Table 2) can be influenced by environmental, horticultural, or biological factors. For example, bitter pit and water core are correlated with orchard factors (water and mineral nutrition) while other disorders (storage scald, Jonathan spot) are more often considered as storage accidents (Bondoux, 1992). Nevertheless, the origin of some physiological alterations is often difficult to know because symptoms can be associated to different causes. For this reason, it is difficult to classify them.

TABLE 2
Characterisation of common physiological disorders
(modified from Bondoux *et al.* 1992)

Disorder	Cause	Correlation with mineral content	Period of apparition
Bitter pit	Cellular collapses	Ca (-), Mg (+), K (+), P (+), N (+)	Orchard and storage
Water core	Cell wall rupture (excess of glycerol)	Ca (-), N (+)	Orchard and beginning of storage
Storage Scald	Production of toxic compounds	Ca (-), N (+)	Storage
Russet	Abnormal growth of the epidermal cells	N (+)	Storage
Jonathan spot	Cellular collapses	Ca (-)	Storage
Deep browning	Senescence, low temperature injury and gas concentration during storage	Ca (-)	Storage

"Bitter pit" usually appears as depressed brown lesions in the skin of fruits (Fig. 5), located mainly on the calyx end of the fruit (Ferguson and Watkins, 1989). Peeling the affected area reveals dry, brown corky flesh. It often appears after harvest, although it can be found in fruits of certain varieties in the orchard when the problem is

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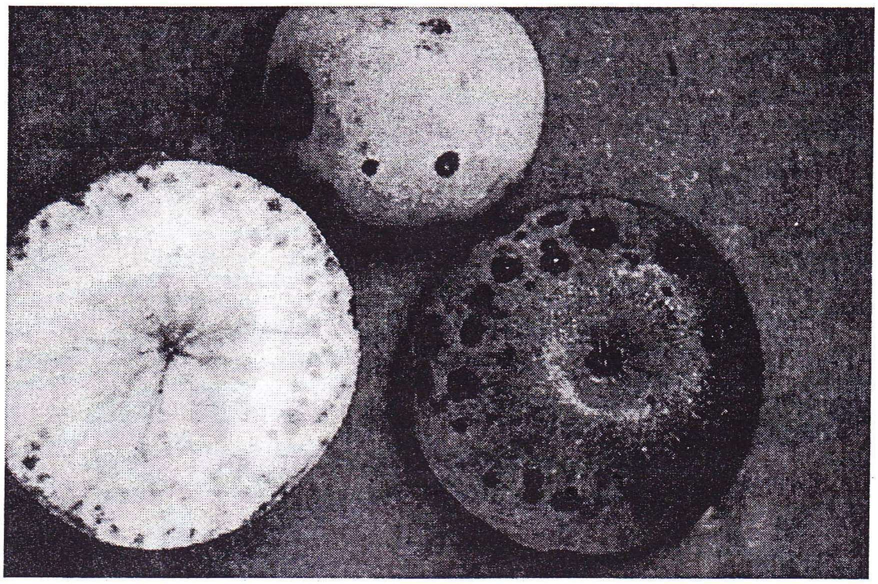

Fig. 5: Bitter pit (provided by P. Creemers, Royal Research Station of Gorsem, Belgium)

severe. Bitter pit is a disorder of apples related to a mineral imbalance within the fruit (Retamales and Valdes, 2000). The incidence of the disorder is related to a deficiency of Ca content in the fruit and, in general, is directly related to magnesium, potassium, phosphorous and nitrogen levels in fruit tissues. Numerous other factors have been associated with bitter pit such as genetic predisposition, fruit size and cropping status, canopy attributes, rootstocks, irrigation and water status, fruit developmental rate and maturity, storage conditions.

"Water core" consists in liquid-soaked tissue mainly around the vascular bundles (Loescher and Kupferman, 1985). Nevertheless, the disorder can also appear in any part of the flesh tissue. In case of severe attack, the tissues turn to a glassy appearance due to the presence of liquid in the intercellular spaces. Analytical comparisons of affected and healthy fruits have shown elevated water content, decreased reducing sugars and higher sorbitol content in water-cored apples. Water core tissue lacks the ability to convert sorbitol to fructose creating the accumulation of toxic compounds such as ethanol and acetaldehyde (Jones and Aldwinckle, 1990). High nitrogen and low calcium fruit concentration can increase the incidence of the disorder. As bitter pit, symptoms can appear in the orchard.

"Storage scald" (or "common scald" or "superficial scald") is a physiological disorder characterized by a brown discoloration of the skin (Kupferman, 1993) (Fig. 6). Only a few layers of cells beneath the skin are affected. Usually, fruit develop scald symptoms after storage when they are exposed at room temperature during a few days. The production of naturally toxic compounds (terpene, α-farnesene) in the fruit peel seems to cause this browning (Jones and Aldwinckle, 1990). Factors influencing the increase of storage scald severity are early harvest, high nitrogen and low calcium fruit content, warm pre-harvest weather, delayed cold storage, high temperature and relative humidity in storage room.

Fig. 6: Storage scald (provided by P. Creemers Royal Research Station of Gorsem, Belgium)

"Russet" symptoms are characterised by cork on the outer surface of fruit (Jones and Aldwinckle, 1990). Apple russet is associated with some environmental conditions, such as high humidity, rain or dew on the fruit, frost. Russet disorder is related to abnormal growth of the epidermal cells. Physical damage of the cuticle (particularly between bloom and 30 days after petal fall) can stimulate too rapid division of underlying epidermal cells, causing the cuticle rupture followed by the cork development. Other factors are also associated with the

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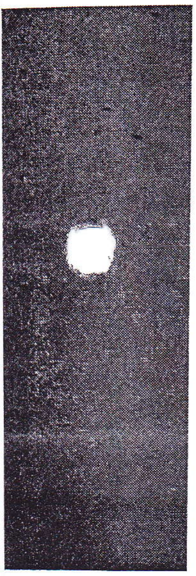

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"Jonathan spot" is often associated with lenticels. Symptoms begin with small brown to black spots (Jones and Aldwinckle, 1990). As the disorder progress, spots coalesce and form irregularly shaped blotches. The spots usually do not penetrate the flesh. The disorder can appear on other cultivars than 'Jonathan' (*i.e.* 'Golden Delicious', 'Idared', 'Newtown'...). The occurrence of Jonathan spot is related with low calcium concentration and storage procedures (slow cooling, high storage temperature).

Finally, "deep browning" of the apple flesh can also appear (Jones and Aldwinckle, 1990). Symptoms are generally characterised by various tanning but some other alterations may be present (cracking, floury fruit,...). These symptoms can be attribute to overmaturity of the fruit, low temperature injury and atmosphere composition (too high concentration of CO_2 eventually linked to a low O_2 concentration).

4. Traditional methods of control

4.1. Control of factors influencing the fruit physiology

A number of practices linked to the control of fruit physiology are designed to prevent or to delay the incidence of post-harvest diseases in apples. These include controlling growth conditions of trees in the orchard, harvesting before the climacteric rise, avoiding mechanical injuries and modifying the environment during pre-storage, storage and transit in order to reduce the rate of respiration.

Strategies for decay prevention must include proper fertilization. As described before, incidence of several physiological disorders can be attributed to improper nutrition (high nitrogen and/or low calcium concentration). For example, bitter pit being linked to the calcium concentration of fruit tissues, its control has been achieved mainly toward supplementing the Ca supply to the fruit *via* pre-harvest foliar applications eventually followed by a post-harvest calcium treatment by dipping, drenching or pressure infiltration (Conway, 1991 ; Conway and Sams, 1983).

Incorrect nutrition, as provided by high nitrogen, makes also fruit more susceptible to some fungal decays. Studies by Sugar (1994) showed that decay was reduced in fruit with relatively low nitrogen to calcium ratios. On the other hand, fruits presenting a high potassium/ calcium ratio reach more rapidly the climacteric point and become subsequently more susceptible to fungal decay. Beyond this indirect effect, higher calcium concentrations in apples inhibit or delay the symptom development by *P. expansum*, *B. cinerea* or *C. gloeosporioides* (Conway, 1991). It was demonstrated that higher calcium content in apple tissue improves the cell wall structure and integrity (Conway, 1987). It was also observed that the inhibition of some pathogens such as *P. expansum*. by Ca was linked to the decreased of polygalacturonase activity produced by the pathogen. To spray trees with calcium chloride during the growing season is thus also recommended to delay fungal decay in stored fruit. In that context, management of the orchard must provide fruit at harvest with high and balanced mineral nutrient contents to have a low risk of disorders and have optimal storage properties.

The evolution of fruit maturity plays an important role in the development of rots. Apples must be harvested before the climacteric rise. Pre-climacteric fruits are usually firmer than mature fruits and more resistant to mechanical injuries occurring during harvest and post-harvest handling before storage. Moreover, the internal resistance of fruits against fungal diseases decreases with maturity (Creemers, 1998).

It is crucial to operate carefully during harvest and post-harvest handling in order to limit mechanical injuries (Herregods, 1990). The ability of wounds to heal plays an important role in the resistance against wound pathogens and is associated to the ethylene production (Sommer, 1989). In apples, as for many others fruits, the healing process is characterized by the construction of a periderm. The generation of that barrier is possible during cell division and extension. After harvest, the fruit is not able anymore to produce such a barrier. Nevertheless, cells around the wound site are able to strengthen their wall by synthesising molecules based on lignin and callose. The optimal conditions to promote such a process (85 % RH and 10°C) are rarely met during harvest and post-harvest treatment and never during storage. In contrary to this, when apples are received in the

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packinghouse, they should be placed into cold storage so that field heat can be removed as quickly as possible. On unwounded fruits, rapid removal of heat has a positive effect on both fruit quality and reduction in storage decay. Room loading and bin stacking procedures should be established to allow the rapid filling of rooms, and excellent air flow for cooling (Kupferman, 1986).

Storage environmental conditions of apples such as moisture, ventilation, temperature and oxygen and carbon dioxide concentrations directly influence the development of physiological disorders (cf. 3) and fungal diseases. High relative humidity and low temperatures were first applied respectively to avoid fruit desiccation and to delay the maturation but these conditions were not enough efficient to control the fruit respiration. Actually, technical progresses allow storing the fruits in controlled atmosphere (CA). Such CA storage rooms are characterized by a low oxygen concentration (2 to 3 %) and a high carbon dioxide content (2 to 5 %). The deprivation of oxygen is supported until a level of 2 % (or sometime less) by numerous varieties (Kupferman, 2001). Atmospheres containing simultaneously very low O_2 (1 to 1.5 %) and CO_2 levels are also employed. These ultra low oxygen (ULO) storage rooms slow down the respiration process and ethylene synthesis, insuring the firmness of the fruits and increasing the storage period of 7 to 9 months (Marcellin, 1990). Novel storage rooms are developed in United-Kingdom, Italy and USA (Kupferman, 2000) where the ethylene produced by apples is eliminated. An increase of acidity, flavour and firmness of 'McIntosh', 'Empire' and 'Golden Delicious' was observed when these varieties were stored in ULO with low ethylene level in comparison with classical ULO. However, it is impossible until now to maintain an extremely low level of ethylene in the storage atmosphere when the volatile compound is produced in high quantity (Kupferman, 2000).

The phytosanitary problems have evolved in parallel with the technical changes of storage. Some physiological disorders are associated to CA or ULO conservation. For example, low oxygen can sometimes induce a loss of flavour followed by an alcoholic flavour generated by anaerobic fermentation. In some varieties, the red area of the skin will turn purple and green areas into bronze. The sensitivity to high level of carbon dioxide is more frequent and linked to the variety, the fruit age and the origin of harvest (Kupferman, 2001;

Marcelin, 1990). The skin of the fruit will be rough and stained with a swonflake pattern on some varieties. The flesh of affected fruit will become brown and in many cases will develop cavities. The core tissue may also turn brown (coreflush). 'Cox's Orange', 'Granny Smith' and 'Elstar' are more sensitive to high CO_2 level than 'Golden Delicious' and 'Jonagold'. In modified atmosphere associated with the control of ethylene level, a decrease of scald was observed on 'Golden Delicious' (Kupferman, 2000).

Low temperature decreases the growth of fungal pathogens. The critical temperature inhibiting the fungal growth depends on the pathogen. Nevertheless, fungal diseases responsible of important economical losses (*B. cinerea*, *P. expansum* and *C. curvispora*) are able to growth at 0°C, apples being stored at slightly higher temperature. CA and ULO conditions frequently inhibit the sporulation of fungi. Fungal growth can be directly decreased in ULO storage. Due to the physiological preservation of apples, the development of latent infections is sometimes sufficiently delayed to allow the commercialisation of the fruits before the occurrence of symptoms. High relative humidity in the storage rooms favours the development of pathogens (Creemers, 1998). Furthermore, poor ventilation around storage containers leads to increased moisture around the fruit and slower cooling times, which can increase the risk of infection.

4.2. Sanitation

Sanitation is a useful tool in post-harvest disease management strategy and consists in reducing, removing, eliminating, or destroying inoculum at the source. In case of post-harvest apples systems, this includes sanitation of field bins, packinghouse water systems, and packing and storage facilities to avoid inoculum of fungal post-harvest pathogens.

Part of the sanitation strategy consists in discarding damaged fruit lying around a packinghouse. A single apple may have billions of fungal spores on its surface that may be redistributed to new infection sites (Kupferman, 1986). It has also been recognized that spore of fungal pathogens accumulate in water systems (Heald *et al.*, 1928). Dump tank water sanitation is critical to reduce spore loads in water because the higher the numbers of spores in dump tank water system, the more infection sites will be inoculated and the greater the risk of decay (Spotts, 1986). The contribution of contaminated field bins to populations in drenches and flume water systems has been established more recently, showing the necessity to also sanitise that material (Sanderson, 2000). Spores produced on decay lesions on fruit in storage can be blown around the rooms by the refrigeration fans and may cause new infection sites. These spores, in addition to those that persist on fruit surfaces and bins, also can contaminate water systems. For the same reason, packing facilities and surfaces must also be sanitised.

In many countries, there is no systematic process for sanitizing field bins even if numerous techniques can be used such as washing with a disinfectant (chlorine, sodium orthophenylphenate or SOPP, quaternary ammonia compounds), pressure washing with hot water, or steam cleaning (Apel, 1989; Kupferman, 1986). Chlorine compounds, quaternary ammonia formulations, and steam are also effective sanitizers for packinghouse line/hard surfaces, including cold storage room surfaces. Chlorine is a biocide also used to treat process water (Apel, 1993). Different salts of chlorine (sodium hypochlorite, calcium hypochlorite, bromochlorodimethyl-hydantoin) are recommended depending on the country legislation. Chlorine should only be used in solutions where the pH (acidity/alkalinity) is around neutral (pH 6.5-7.5) (Kupeferman, 1986). The biocide activity of these salts is also directly influenced by the temperature, the concentration of the product (50 to 100 ppm of free chlorine), and the amount of dirt in the water (Kupeferman, 1986; Eckert and Ogawa, 1988).

Ozone can also take part of an overall sanitation program to disinfect cold storage rooms and water systems (Tukey, 1993). Ozone, a very powerful oxidizer, can be generated through the use of ultraviolet (UV) light or electricity. The latter method, called corona discharge, is the most common way to generate ozone in large quantities. Ozone presents the advantage to have a short half-life time (15 minutes). For this reason, it needs thorough mixing to be effective as a water disinfectant treatment. Disadvantages of its use are its corrosive activity to many common materials, like rubber and mild steel and its human toxic effect.

In the mid 1990's, studies showed that acetic acid vapour was very effective in killing fungal spores of post-harvest pathogens (Sholberg and Gaunce, 1995). These results indicated that acetic acid vapour could be a possible alternative, or could be used in conjunction with chlorine for disinfecting fruit.

4.3. Chemical treatments against post-harvest diseases

Until now, control measures against post-harvest diseases are mainly based on the protection of fruits from pre- and post-harvest infection with pre- and post-harvest treatments. These treatments aim at depositing enough quantity of active ingredients on fruits to insure their protection against diseases during the total period of storage.

For pre-harvest treatments, the strategy consists in several applications of fungicides to prevent post-harvest fungal diseases. The active ingredients used against post-harvest pathogens belong to benzimidazole or MBC (benomyl, carbendazim, thiophanate-methyl, thiabendazole or TBZ), phenylcarbamate (diethofencarb), phtalimide (captan), dithiocarbamate (thiram, ziram), sulfamide (tolylfluanide) (Creemers, 1998; Eckert and Ogawa, 1988; Environmental Protection Agency, 2002; Franclet, 1994; Jijakli *et al.*, 1999; Locke *et al.*, 2002; Sanderson, 2000). The number of active ingredients may vary per country depending on its legislation. Examples are presented in Table 3.

TABLE 3
Registered conventional active ingredients for pre-harvest applications in Belgium, France, UK and USA

Fungicide group	Active ingredient	Diseases (partially) controlled * against major diseases	Countries
Benzimidazole (MBC)	Benomyl	B, P and G	USA
	Carbendazim	B, P and G	Belgium, UK and USA
	Thiabendazole	B, P and G	Belgium, USA
	Thiophanate methyl	B, P and G	Belgium, France, UK, and USA
Dithiocarbamate	Thiram	B, P and G	Belgium, UK and USA
	Ziram	B, P and G	USA
Phtalimide	Captan	B and G	Belgium, UK, and USA
Sulfamide	Tolyfluanide	B	Belgium and France

* : Expected control in absence of resistant strains of the pathogen
B = *B. cinerea*, P = *Penicillium* spp., G. = *Gloeosporium* spp.

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The number of pre-harvest fungicidal treatments against post-harvest diseases ranges generally around 3 to 4 in orchards presenting a high density of pathogens (*B. cinerea*, *Gloeosporium* spp. and *Penicillium* spp.). For example, six weeks before harvest, a benzimidazole fungicide is applied in Belgium, followed by a treatment with captan or thiram, an application of benzimidazole 14 days before picking, then comes a last spraying with tolyfluanid one week before harvest (Creemers, 1998). In France, the most usual programme of treatments includes two sprays with tolyfluanide and one application of an active ingredient belonging to the benzimidazoles.

Post-harvest treatments are applied to control both physiological disorders and fungal diseases occurring after harvest. When apple varieties such as 'Delicious' are highly susceptible to the physiological disorders (storage scald), it is recommended to treat them with an antioxidant, either diphenylamine (DPA) or ethoxyquin (Sanderson, 2000 ; Biggs and Rosenberger, 2001).

The number of active ingredients authorized for post-harvest applications may also varies from one country to another (*see* Table 4). The sole active ingredient registered in France is TBZ. Currently,

TABLE 4

Registered conventional active ingredients for post-harvest applications in Australia, Argentina, Belgium, France, UK and USA

Fungicide group	Active ingredient	Diseases (partially) controlled *	Countries
Benzimidazole (MBC)	Benomyl	B, P and G	Argentina,
	Carbendazim	B, P and G	Australia, Argentina, and UK
	Thiabendazole	B, P and G	Australia, Argentina, Belgium, France, and USA
	Thiophanate methyl	B, P and G	Argentina and UK
Dithiocarbamate	Iprodione	B, P and G	Argentina and Australia
Imidazole	Imazalil	B and P	Argentina and Belgium
Phenylamide + MBC	Metalaxyl + carbendazim	B and G	UK
Phtalimide	Captan	B, P and G	UK and USA

* : Expected control in absence of resistant strains of the pathogen
B = *B. cinerea*, P = *Penicillium* spp., G. = *Gloeosporium* spp.

only two conventional post-harvest fungicides (captan and TBZ) are allowed for use on apples in the United States (Sanderson, 2000). Many other fruit producing countries can use fungicides that are not registered in the United States or in France. In Australia, the benzimidazole fungicides (carbendazim and TBZ) and iprodione are registered. Benomyl, TBZ, carbendazim, thiophanate methyl, as well as iprodione and imazalil are authorised for post-harvest application in Argentina (Sanderson, 2000). Other examples are listed in Table 4. In that frame, fruits from one country should be segregated and treated for the export market to which the fruit is assigned, as it is already applied in both Argentina and Chile.

Benzimidazole family is mainly used against *B. cinerea*. These fungicides are applied since 1970 (Eckert and Ogawa, 1988). Certain strains of *Penicillium* or *Botrytis* are resistant to all the benzimidazole family (Biggs and Rosenberger, 2001). This phenomenon was foreseeable due to the intensive use of these fungicides against the same pathogens on other crops (Creemers, 1987). The first resistant strains were detected in late 1970's in storage rooms. The resistance seems to be persistent when no benzimidazole is applied anymore. Despite the restriction of their use against post-harvest diseases and their interdiction against scab or powdery mildew in some countries, resistant strains of *B. cinerea* and *Penicillium* sp. are still present in most cold chambers (Franclet, 1994; Prusky *et al.*, 1985 ; Rosenberger, 1991; Vinas *et al.*, 1991). Recent work revealed that between 30% to 50% of isolates of *P. expansum*, recovered from drenches, were resistant to TBZ (Sanderson, 2000). Furthermore, growing number of *Gloeosporium* spp. resistant strains to benzimidazole fungicides appeared during the last years (Bondoux, 1992). In the mid-1980s, most of the fungicide resistant strains of *Penicillium* and *B. cinerea* were unusually sensitive to DPA (Biggs and Rosenberger, 2001). Thus, the combination of DPA and a benzimidazole fungicide provide good control against both pathogens though the late 1980's. However, about 2 % of the *P. expansum* strains recovered from apple storages were resistant to both DPA and TBZ, even in the mid-1980's. It appears that these strains have gradually increased in importance and should be at least partially responsible for declining effectiveness of post-harvest treatments in some countries (Biggs and Rosenberger, 2001).

In order to avoid the development of fungicide-resistant strains, the anti-resistance strategies during pre- and/or post-harvest treatments should consist in alternating unisite fungicide family with different modes of action and associate multisite fungicides such as tolyfluanide, thiram or captan (Creemers, 1998). In USA, one current approach for controlling TBZ-resistant strains of *Pencillium* is to add captan to the post-harvest treatment solutions (Biggs and Rosenberger, 2001). Tolyfluanid has a large spectrum of action and its use for pre-harvest application is advised in an anti-resistance strategy in France and in Belgium (Creemers, 1998). On the other hand, a negative cross-resistance is usually seen between the fungicide families benzimidazole and phenylcarbamate. Sumico, a product based on carbendazim and diethofencarb and commercialised in Belgium, is used to acquire a predominant role in the control of post-harvest diseases of apples (Creemers, 1998). However, this product is not registered anymore in Belgium.

Indeed, governmental policies of several countries are restricting the use of fungicides or are reassessing the registration dossier of widely used molecules (Gullino and Kuijpers, 1994; Ragdsdale and Sisler, 1994; Wellings, 1996). Because countries are currently revising the (re)registration of active ingredients, Tables 3 and 4 should be used as a general reference. The use of vinchlozoline is already restricted during the flowering period in Europe. Application of captan is not allowed anymore in Germany, while the period between its last application in the orchard and the harvest was enlarged in other European countries. The possible deregistration of benzimidazole family is also discussed at European community level. Benomyl is already forbidden and the carbendazim deregistration could follow. In USA, some fungicides have already lost their post-harvest registrations, such as benomyl and iprodione. Four commonly used post-harvest chemicals (captan, TBZ but also DPA and SOPP) were recently scheduled for a tolerance reassessment by the U.S. Environmental Protection Agency (EPA) (Warner, 1998). An additional ten-fold safety factor may be applied for chemicals used on foods that are common in the diets of infants and children. If captan and DPA were reregistered, the current status of the other molecules remains unclear. EPA will reassess other active ingredients in the near future.

Apples being considered as a minor crop for agro-chemical companies and the registration process being expensive in comparison of the potential market size, there is no specific development of novel fungicides against post-harvest diseases on apples. However, novel synthetic fungicides, which were recently developed against diseases on major crops, are being evaluated against apple rot agents (Sanderson, 2000). Trifloxystrobin, belonging to the strobilurin family, is already registered in Switzerland for pre-harvest treatments against Gloeosporiodes group. But the pre-harvest use of trifloxystrobin in Europe will be probably limited because the main targeted pathogens are apple scab and mildew and resistance phenomenons were already detected for both fungi (Creemers, personal communication). Fludioxonil (pyrrole), fenhexamid (anilide), tebuconazole (triazole) and cyprodinil (anilino-pyrimidine) are being tested in several European countries and USA. Fludioxonil has shown broad spectrum efficacy against post-harvest pathogens, whereas fenhexamid is very effective against *B. cinerea*, *Monilia* spp. but not against *Penicillium* spp. or *Gloeosporium* spp. (Sanderson, 2000). Tebuconazole and cyprodinil are also efficient against *B. cinerea*. Some of these active ingredients are already registered to treat other fruits than apples against storage diseases but the authorisation of their use against post-harvest diseases on apples might take a couple of years and it is still not clear if they will be used for pre- or post-harvest treatments.

5. Integrated control and organic production

The consumer reluctance to chemical residues in food and the public concern for human and environmental safety have promoted both the restrictions of fungicide uses and the emergence of Integrated Fruit Production (IFP) and organic orchards.

The approach to Integrated Plant Protection in sustainable production system was described by Boller *et al.* (1999). All available prophylactic (indirect) plant protection measures must be applied before direct control measures are used. The decision of the application of direct control measures must be based on economic thresholds, and risk assessments. Priority is given to natural, cultural, biological, genetic, and biotechnical methods of disease control and the use of agrochemicals must be minimized (Cross, 2002). The IFP market has considerably increased this last decade and reached 53% of the surface of European countries/regions in 1997 (Dickler *et al.*, 1999). The producers of IFP are allowed to treat their orchards with a limited number of products classified according to their efficacy, selectivity and environmental safety (Cross, 2002). Until now, the Integrated Plant Protection concept was mainly developed with success for the protection against insects and pre-harvest fungal pathogens such as apple scab or powdery mildew. However, the IFP guideline imposes that post-harvest fungicide treatments may only be used where suitable non-chemical methods are not available. Furthermore, post-harvest treatment with synthetic, non-naturally occurring anti-oxidants for control of superficial scald and other disorders is not allowed in Europe. The search and the development of alternatives methods for the control of post-harvest diseases is an important challenge for the further development of this integrated approach. Furthermore, post-harvest treatments are authorized only on fruits with a high probability of rotting. Unfortunately, only few predictive models have been published such as a model for bitter pit risk on Golden (Sio *et al.*, 2001) or a model for rot risk on Cox in England (Berrie, 2000).

The organic production market remains a marginal sector of activity (less than 3 % in Europe and in USA). No treatment against post-harvest rots is applied in European organic orchards. Authorized fungicidal treatments (copper and sulfur-based products) are only active against scab. Copper could be also deregistered in Europe in 2004 due to its toxicity on the soil fauna. As a consequence, the persistence of this organic sector relies on the finding of new control methods.

6. Emerging technologies of control

The progressive loss of fungicide effectiveness due to selection of resistant isolates of pathogens, the growing scarcity of fungicides devoted at post-harvest situations and the public pressure concerning the risk of residues on fruits have promoted the search for alternative methods. Several novel approaches are emerging as possible alternatives to synthetic fungicides, including biological control agents (BCAs), application of natural biocides, induction of natural defence mechanisms of harvested products, and genetic resistance (Falik *et al.*, 1995; Janiziewicz and Korsten, 2002; Jijakli *et al.*, 1999; Tukey, 1993 ; Wilson *et al.*, 1994).

6.1. Biological control

Biological control is generating a great enthusiasm to play a role in sustainable agriculture although the relevance of BCAs in plant pathology appears limited until now. If everybody recognises the existence of natural phenomena of microbial antagonism, the question is to know how to manipulate the occurring antagonistic micro-organisms to achieve a reliable and effective strategy of disease control meeting the requirements of the market. The post-harvest phase is particularly suited for the application of biological control methods (Jijakli *et al.*, 1999). The application sites are limited to the fruits and the harvested commodities are of high value. Furthermore, variation of temperature, relative humidity, or gas composition can be minimized in storage room allowing the selection of micro-organisms better suited for one set of particular conditions.

Before becoming an economically feasible alternative to chemical control, BCAs have to satisfy different requirements related to biological, technological and toxicological properties.

The different steps of research and development for a successful strategy of disease control with BCAs are represented in Fig. 7. These steps are all essential and complementary to the others. An "ideal antagonist" should have the following characteristics (Jijakli *et al.*, 1999): effective at low concentrations in several post-harvest host pathogen combinations; able to survive under adverse environmental conditions such as low temperatures and controlled atmospheres prevailing in storage facilities; amenable to inexpensive production and formulation with a long shelf life; easy to dispense; compatible with commercial handling practices; genetically stable; non pathogenic for the consumer and for the host commodity.

There are numerous examples in the literature of biocontrol agents, active against wound pathogens (mainly *B. cinerea* and *P.*

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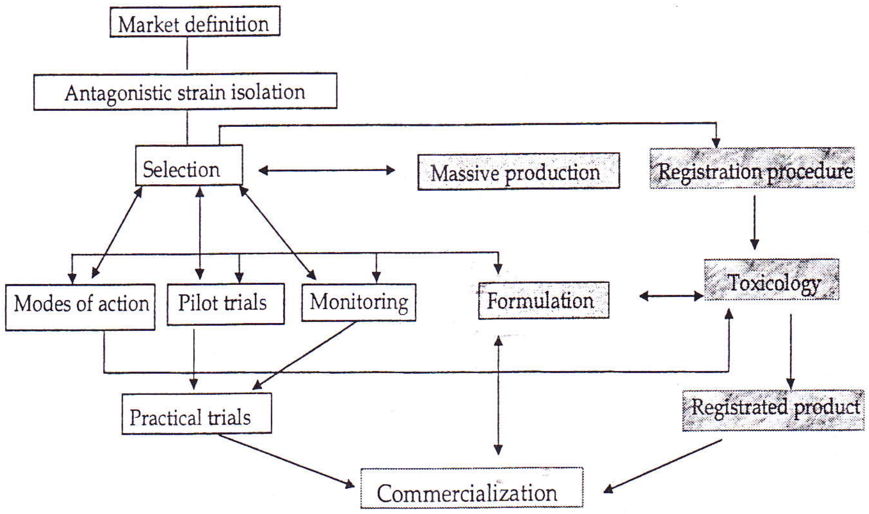

Fig. 7: Steps leading to the practical use of BCA's (modified from Jijakli *et al.*, 1999)

expansum) of apple fruits (for a review *see* Janisiewicz and Kosten, 2002; Jijakli *et al.*, 1999; Wilson and Wisnieswski, 1994), but to date only three have reached the market. Two biocontrol products have been commercialised in USA and are used as post-harvest treatments on apples for control of wound diseases: Biosave™ (*Pseudomonas syringae*, Esc-11) by Ecoscience Corp and Aspire™ (*Candida oleophila*, I-182) by Ecogen Inc. Yield Plus™ (*Cryptoccoccus albidus*) constitutes the third biocontrol product against post harvest diseases on pome fruits and is sold in South Africa by Anchor Yeast, but little has been reported about that last product (Janisiewicz, and Kosten, 2002). In Europe, no biocontrol products active against post-harvest diseases of apples are available until now. However, some biological control agents such as *Candida oleophila* strain O (Jijakli *et al.*, 1999) or *Candida sake* strain CPA-1 (Usall *et al.*, 2000) are in the development phase and might reach the European market soon. There are several bottlenecks explaining the low number of registered biopesticides for use on apples against post-harvest diseases and the moderate success of such commercialised products. Among them, the lack of reproducibility and reliability of BCAs efficacy when they are used in practice constitutes the major limiting factor.

The improvement of the biocontrol has been accomplished by several approaches such as (i) the combination of several BCAs against a same pathogen (Nunes *et al.*, 2002), sometimes on basis of niche differentiation (Janisiewicz, 1996); (ii) the enlargement of the spectrum of controlled diseases with mixtures of compatibles BCAs (Janisiewicz 1988; Janisiewicz and Bors, 1995) or selection of particular micro-organisms controlling a panel of pathogens (Janisiewicz *et al.*, 1996); (iii) the manipulation of the environment in which the BCA will operate such as the addition of nutrients (Janisiewicz, 1994; Jijakli *et al.*, 1999); (iv) the combining of the BCA treatment with low doses of fungicides (Chandgoyal and Spotts, 1996), organic (El-Ghaouth *et al.*, 2000, Jijakli *et al.*, 2003) and inorganic additives (Jijakli *et al.*, 1999; Nunes *et al.*, 2002), or physical treatments (Leverentz *et al.*, 2000; Jijakli *et al.*, 1999); (v) the suitable production and formulation of the antagonists (waxes, salts,....) (Abadias *et al.*, 2001); (vi) the physiological improvement of the BCA's (Texido *et al.*, 1998).

More recently, pre-harvest treatments with BCAs were also assessed against post-harvest wound pathogens on fruits with some success (Ippolito and Nigro, 2000 ; Jijakli *et al.*, 2003). Antagonists are applied few days before harvest in order to precolonize the fruit surface. It is expected that antagonistic strain will colonize the wounds created during harvest prior pathogen colonisation. In that approach, many of the advantages of post-harvest application are lost (Janisiewicz, and Kosten, 2002) and environmental fate studies must be undertaken to assess the ecological suitability of a particular strain under unfavourable factors like UV light, changes in temperature, humidity and nutrient availability, *etc.* (Jijakli *et al.*, 2003).

The science and practice of biological control agents is still in its infancy compared to fungicidal treatment, even if the progress made in this area during the past decade and a half has been remarkable (Janisiewicz, and Kosten, 2002). In the long term, basic information on the genetically determined factors that control survival, colonisation, effectiveness in the field and storage and properties of mass production are required to overcome the random process of selection and to facilitate the practical development of such a method (Jijakli *et al.*, 1999). This information will help in finding how to (i) enhance the protective action of BCAs, (ii) protect the viability and the performance of BCAs under unfavourable environmental conditions, (iii) ensure a

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good stability of the product during storage prior to application, and (iv) provide a user-friendly product that is easy to apply.

6.2. Natural biocides

Plants produce a large number of compounds (constitutively or after induction) with potential activity against micro-organisms. Among these compounds, some extracts and essential oils from various plants revealed a biocide action. Recently, large *in vitro* studies on the control of fruits post-harvest pathogens have been carried out (Daferera *et al.* 2000; Wilson *et al.*, 1997) and showed fungicidal or fungistatic activity. Three essential oils produced by *Cymbopogon martinii*, *Thymus zygis* and *Eugenia caryophyllata* were the most efficient inhibitors of *in vitro B. cinerea* spore germination (Wilson *et al.*, 1997). Thymol and citral, two essential oils, showed *in vitro* a high inhibitory effect against *P. expansum* growth (Venturini *et al.*, 2002). The use of carvone (mint extract) or eugenol (clove extract) by dipping harvested apples controlled the development of post-harvest fungal pathogens on these fruits (Bompeix *et al.*, 2000). However, the high concentrations required to obtain an acceptable protective level could constitute an economical barrier for practical application.

Some volatile aromatic components produced by fruit during ripening such as acetaldehyde also showed fungicidal or fungistatic activity. The resistance of strawberries to some pathogens is partially attributed to a high production of acetaldehyde (Wilson and Wisniewski, 1989). Some volatile compounds present a low toxicity for mammals (*see* Mari and Guizzardi, 1998 for a review) and might be promising in fumigation in cold storage or in packaging.

Other natural products are derived from other organisms. Chitosan is a high-molecular weight polysaccharide derived from alkaline deacetylation of chitin, an animal component (de Capdeville *et al.*, 2002). Chitosan controlled partially the development of post-harvest decays such as *B. cinerea* or *P. expansum* (de Capdeville *et al.*, 2002). This action is the result of direct toxic effect on pathogens, indirect effect on fruit senescence and stimulation of fruit resistance by increasing chitinase and β-1,3-glucanase activities (de Capdeville *et al.*, 2002).

6.3. Intensification of natural defence mechanisms

Fruits contain a multitude of highly coordinated defensive mechanisms that naturally protect them from invading micro-organisms (Forbes-Smith, 1999). Accumulation of phytoalexins, modification of the structural barriers, and synthesis of antifungal hydrolases such as chitinase and β-1,3-glucanase are part of the various natural plant defence strategies against microbial attack that should be enhanced. This objective can primarily be attained by slowing down the ripening process (*i.e.* by manipulating storage conditions, *see* 4.1) in order to maintain constitutive and inducible defence responses. Further induction of resistance against post-harvest rots has also been observed after physical, chemical or biological treatments.

Physical methods of control of post-harvest diseases on apples are promising because they leave no residues in/on the fruit. The beneficial effect of low doses of UV-c occurs in several post-harvest commodities including stone, pome and citrus fruit (Stevens *et al.*, 1996). The development of disease resistance after such a treatment coincides with the accumulation of phytoalexins in host tissue such as lemon, carrot roots or grapes (Forbes-Smith, 1999). In grapefruit, UV-c light treatment is correlated with an increase of phenylalanine ammonia-lyase and peroxidase activities (Droby *et al.*, 1993). Wilson *et al.* (1997) developed an apparatus that delivers UV-c light. Its application on a processing line significantly reduces post-harvest decay in apples. UV-c light also directly affects the pathogen as shown on conidial survival of *P. expansum* (Valdebenito-Sanhueza and Maia, 2001). The optimal dosage of UV-c light depends on cultivars and physiological age (Forbes-Smith, 1999). Application of too high doses may increase the susceptibility of the host tissue to pathogen invasion.

Pre-storage heating of post-harvest commodities may be also advantageous as a natural control strategy by directly inhibiting pathogen growth (Jijakli *et al.*, 1999) but also by accelerating natural resistance of host tissue. In case of apples, the stimulation of wound healing process seems to be partially responsible for the resistance against post-harvest diseases. Thermal treatments (bath at 45°C, 10 min) were susceptible of reducing latent infections (Jijakli *et al.*, 1999) and disinfect the surface of the fruits but does not offer a long-term protection (microbiological vacuum). However, this treatment may increase the susceptibility to *P. expansum*, *B. cinerea* or *Alternaria* sp. (Edney and Burchill, 1967, Jijakli *et al.*, 1999).

Several chemical compounds such as chitosan (cf. 6.2), calcium (cf. 4.1), harpin or acibenzolar have shown their ability to induce resistance in harvested apple fruit. For example, harpin, a peptide produced by the plant pathogenic bacterium, *Erwinia amylovora* (Burrill) Winslow *et al.*, induced resistance in apple fruit against blue mould and the resistance depended on harpin concentration and the interval between treatment and inoculation (Capdeville *et al.*, 2002). Nevertheless, the protective level due to these compounds doesn't reach the level of conventional fungicides. Finally, the possibility to induce resistance has also been observed with antagonistic yeast's on apples infected by *B. cinerea* or *P. expansum* (Capdeville *et al.*, 2002; El Ghaouth *et al.*, 2000).

6.4. Genetic resistance

Spotts *et al.* (1999) have recently shown differences among apple varieties through a study of their susceptibility to four post-harvest fungal pathogens and through some physical properties (force to break epidermis, sinus opening). Natural sources of resistance can then be found, but selection programs are time-consuming.

Insights into genes involved in ripening, senescence, defence reactions, respiration, as well as into expression of factors triggering genes after harvest or at stage where the host is more sensitive, lead to good hope of developing resistant plants by traditional breeding or genetic transformation (*see* Arul, 1994, for a review). For example, considerable progress has been made on ethylene regulating fruit ripening in association with its perception and signal transduction and gene expression (Jiang and Fu, 2000). ACC synthase and ACC oxidase, two proteins involved in the ethylene regulation, have been characterized and their genes cloned from various fruit tissues. The properties and functions of ethylene receptors are also being elucidated. As the apple sensitivity to post-harvest diseases is partially linked to fruit physiology, the prospects of ethylene regulating fruit ripening associated with post-harvest life extension should be promising.

The use of foreign genes are also envisaged and gave mitigated results in the following example (pre-harvest field): a line of transgenic apple tree overexpressing an endochitianse (CHIT42) form *Trichoderma harzianum* Rifai proved more resistant to *Venturia inaequalis* (Cook) Wint. but showed a reduced vigor (Bolar *et al.*, 2000). Transgenic broccoli expressing the same enzyme showed reduced sensitivity to *Alternaria brassicola* (Schweinitz) Wiltshire (Mora and Earle, 2001). However, little attention has been paid until now to apple post-harvest diseases.

7. Conclusions

After analysing the current status of post-harvest disease control, it is evident that there is no single solution to such a complex problem. The control of factors affecting the fruit physiology with orchard operations and post-harvest handling practices, the sanitation and the application of synthetic fungicides in pre- and post-harvest treatments are the primary means of controlling post-harvest diseases for conventional IPM programs. However, the presence of chemical residues in food, the development of fungicide-resistant strains of post-harvest pathogens, the deregistration of standard fungicides have generated interest in the development of alternative methods.

Some alternative methods such as natural biocides, BCAs, physical or chemical treatments to induce fruit defence mechanisms, seem promising. BCAs are particularly suited for post-harvest applications against wound pathogens and some of them are already available on the US market. However, the review of emerging methods including physical, chemical and biological treatments demonstrate that in many situation and primarily when the disease pressure is relatively high, the level of protection by the use of a single alternative method rarely reached the one obtained by conventional synthetic fungicide application (if the problem of fungicidal resistance is absent). The complete replacement of the chemical pesticides by one alternative method is then not a reasonable goal. In contrary to this, each method has to be considered as a tool to be used in integrated control strategies. A more realistic scenario would see alternative techniques being used in association with limited quantities of agro-chemicals, as well as efficient management and handling practices to combat diseases in harvested apples. This novel IPM approach must take into account the compatibility of the different treatments, particularly between antagonistic micro-organisms and chemical/physical techniques. That more complex approach is already evaluated in different countries (Biggs *et al*., 2000; Habib *et al*., 2001) and will probably emerge as novel IPM recommendations.

Finally, more attention should be paid to the genetic resistance approach and to the development of reliable forecast systems for post-harvest diseases. The choice of an IPM program should be selected in relation with the predicted disease pressure. The use of synthetic fungicides might be avoided in case of low pathogen pressure. These further studies will help in obtaining a global integrated control strategy to manage post-harvest diseases on apples.

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