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Types of paper: Review Articles

Ethylene control in cut flowers: classical and innovative approaches

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Key words

Post harvest, nanosensors, nanocomposites, nanocatalyst, nanoparticles, nanosponges,

Highlights

- Potential applications of nanotechnology in ethylene control for cut flowers
- Nanoparticle-based sensors for detecting ethylene throughout the distribution chain
- Nanocomposites as scavengers for ethylene removal in active packaging
- Nanocatalysts to promote ethylene catalytic degradation in the warehouse
- Nanoparticles and nanosponges as carriers of drugs for ethylene action inhibition

Abstract

Ethylene-mediated premature floral senescence and petal or flower abscission affect postharvest longevity of several species used as cut flowers. Exposure to exogenous or endogenously produced ethylene can be controlled in several ways. These include the use of ethylene biosynthesis inhibitors or ethylene action inhibitors, and ethylene removal technologies. In addition, genetic modification can be very effective in controlling ethylene synthesis and perception. We review here the potential for applications of nanotechnology to control ethylene levels and postharvest management in the flower industry. Already nanosponges have been shown to enhance efficacy of the ethylene inhibitor, 1-MCP, in several flower species. In carnation, 1-MCP included in nanosponges also allowed better control of *Botrytis cinerea* damage. However other applications are also considered based on successes in the use of this technology to increase agricultural production and decrease postharvest waste. Nano-metal based sensors could be used for detection of ethylene in the store and to label the product along the distribution chain. Furthermore, nanocomposites could be included as scavengers for ethylene removal in active packaging, and nanocatalysts could promote ethylene catalytic degradation in the warehouse. Nanoparticles could also be introduced into a new generation of packaging to control effects of gases and UV, and increase strength, quality and packaging appearance. This review highlights recent results on the use of nanotechnology *sensu lato* and potential application for cut flower vase life improvement, focusing on ethylene control strategies.

Contents

2.2 Nanoparticles and nanoporous materials for ethylene action inhibition

3. Conclusions

1. Introduction

Postharvest performance is a key factor in the commercial value of cut flowers. Although external quality criteria such as appearance, colour and uniformity, are the major variables that influence the consumer's decision to purchase cut flowers, their longevity is fundamental to convince the consumer to re-purchase them (Reid and Jiang, 2012). As a fresh commodity and because of their extreme sensitivity, cut flowers are vulnerable to large postharvest losses. In addition to developmental senescence, cut flowers are also subject to leaf discoloration, premature wilting, and disease from moulds and fungal pathogens, An integrated approach is therefore adopted to maintain quality throughout the distribution chain to reduce water loss (e.g. avoiding high temperatures), control disease (such as *Botrytis* and *Alternaria*) and to limit cut flower ageing (avoiding prolonged cold storage).

Advances in postharvest science and technology aim to provide information for the horticultural industry to enable them to supply attractive and long-lived flowers to consumers. Indeed in the last ten years substantial progress in postharvest technologies has been achieved including novel packaging, storage and transport systems, pest and disease control for market access, senescence control, supply chain optimization, and track and trace systems to ensure delivery of premium quality products to markets (Toivonen, 2007; Michailides and Manganaris, 2009; Sharma, 2010). Chemicals are used extensively in modern agriculture in order to improve yield and quality. However, their use poses environmental and public health concerns. Many chemicals that affect ethylene synthesis or its action, which are currently in use to extend the shelf life of flowers, may be soon banned due to their environmental impact. Over the last decades, environmentally and health-friendly production methods and conscientious use of resources have become crucial for reaching the goal of more sustainable plant production. techniques and systems need to be developed. Thus further progress will require an integration of available bio-, info- and nano-technologies through a systems biology approach.

1.1 Role of ethylene in floral senescence

Ethylene is a simple molecule composed of two carbon atoms symmetrically linked to by a double bond and it naturally occurs in gaseous form. It is, furthermore, a plant growth regulator involved in the regulation of a wide range of different physiological processes, including germination, growth, floral initiation and opening, both leaf and floral senescence as well as organ abscission and fruit ripening (Yoo et al., 2009).

1.1.1 Ethylene as an endogenous and exogenous regulator

Floral lifespan is often terminated by the abscission of petals that are still turgid, or by petal wilting or withering. In many species, these processes are regulated by the plant growth regulator, ethylene (van Doorn, 2001; van Doorn and Woltering, 2008) through changes in endogenous levels. Plant tissues synthesize small amounts of ethylene (0.1-0.2 µl Kg^{-1} h^{-1}; Martínez-Romero et al., 2007). However ethylene production changes during plant development and in relation to physiological status (Yang and Hoffman 1984).

In many species exogenous ethylene can also accelerate floral senescence. Ethylene is produced by many plant tissues (Gane, 1934) and other sources, including bacterial and fungal fermentation processes, and pyrolysis of hydrocarbons, which releases ethylene as a component of air pollutants (Cape, 2003), all of which can thus affect the longevity of cut flowers in the horticultural supply chain. Ethylene is biologically active at very low concentrations (nl-µl l^{-1}), but there are significant differences in ethylene sensitivity between species and even cultivars of the same species (Serek et al., 2006b; Scariot et al., 2008). A detailed classification of flowers based on ethylene sensitiveness is reported by van Doorn (2001).

1.1.2 Plant species: sensitivity and effects

Responses to ethylene vary widely according to the species (Reid and Wu, 1992) although they are often consistent within either families or subfamilies (van Doorn, 2001). Ethylene-sensitive species include a number of important cut flowers. For example petals of orchids (*Phalaenopsis*), *Hibiscus* (Çelikel and Reid, 2002), and carnation (*Diathus caryophyllus*) (Serek et al., 1995a,b) wilt in response to ethylene. In other species, such as *Antirrhinum majus*, *Rosa hybrida* (Serek et al., 1995a), and wax flower (*Chamelaucium uncinatum*) (Macnish et al., 2000), ethylene induces petal or flower abscission.

Ethylene sensitive flowers can be classified into three types (Kumar et al., 2008). First, those like carnation and petunia where senescence is regulated by an increased amount of ethylene production either with ageing or following pollination (Serek et al., 1995a). Second, like cyclamen, which only become sensitive to ethylene and produce increased amounts of the hormone when they are pollinated (Halevy et al., 1984). Third, like rose, which are sensitive to ethylene upon flower bud opening but do not produce elevated amounts of ethylene as they age (Kumar et al., 2008).

As well as accelerating petal senescence and deterioration, ethylene (either endogenous or from an external source) can induce other undesirable physiological disorders to vegetative and flowering organs during postharvest storage of cut flowers both in monocotyledons and dicotyledons including pathogen susceptibility (McKenzie and Lovell, 1992; van Doorn, 2001). For example, *Botrytis cinerea* is one of the most significant postharvest fungal pathogens causing losses in ornamental plants. Disease caused by this fungus has been shown to be enhanced by the presence of ethylene in rose and carnation (Elad, 1988; Seglie et al., 2012). However, depending on the type of pathogen and plant species, the role of ethylene can be dramatically different. Indeed plants deficient in ethylene signaling may show either increased susceptibility or increased resistance (Elad, 1988).

Thus data on ethylene sensitivity of cut flower species is important for predicting effects of exposure during the supply chain such as mixed storage and transport of flowers with fruit species. It is also needed to evaluate the appropriateness of treatments to reduce ethylene production or exposure and to inform breeding programs aimed at improving flower vase life.

1.2 Ethylene control strategies

Ethylene biosynthesis, perception, signal transduction are well-documented as well as is its regulation at biochemical and genetic levels (reviewed in Wang et al., 2002). This knowledge has been used to develop different strategies to reduce ethylene production or inhibit its action (either with new cultivars or vase-life treatments), and in turn to prolong flower postharvest performance.

Premature senescence and abscission caused by exposure to exogenous or endogenous ethylene can be mitigated in several ways (Figure 1) including, ethylene biosynthesis inhibitors, ethylene action inhibitors and ethylene removal technologies (reviewed in Martínez-Romero et al., 2007). Genetic modification is also a very effective way of controlling ethylene synthesis and perception. Attempts to obtain plants with both reduced endogenous ethylene biosynthesis or a reduced ethylene sensitivity have been reviewed by Serek et al. (2006b).

1.2.1 Genetic strategies

Changes in gene expression during petal senescence have been studied through transcriptomics of a number of model flowers (e.g., *Petunia*, *Arabidopsis*) and cut flower species (e.g., *Alstroemeria*, *Dianthus*, *Iris, Sandersonia*) (Rogers, 2013). In species where petal senescence is ethylene-sensitive groups of genes can be identified that are ethylene regulated, comprising transcription factors, genes encoding for enzymes in the biosynthetic pathway for ethylene production, ethylene receptors and ethylene signalling and responsive genes (Rogers 2013).

Ethylene biosynthesis is primarily regulated by 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO) and an early success by the company Florigene in delaying carnation floral senescence was through antisense down-regulation of ACO (Savin et al., 1995). This success was closely followed by down-regulation of ACO in other flower species such as begonia (Einset and Kopperud, 1995) and torenia (Aida et al, 1998). Down-regulation of the ACS gene in carnation also reduced ethylene production (Kiss et al., 2000). Use of antisense sequences in Petunia for ACO and ACS, derived heterologously from broccoli also delayed floral senescence (Huang et al., 2007) showing that the approach can be used more broadly. However, these strategies have no effect when flowers are exposed to exogenous ethylene, as can occur during transit and marketing.

A more effective approach to protecting flowers from exogenous ethylene in the supply chain is therefore to focus on ethylene perception. Ethylene perception occurs through a well-conserved signalling pathway and the receptor is encoded by a family of five genes: *ETR1, ETR2, EIN4, ERS1* and *ERS2* (Yoo et al., 2009). Again an early discovery was that expression of a mutated *ETR1* gene from Arabidopsis (*etr1-1*) disrupts ethylene signalling in a wide range of heterologous species (Bleecker et al., 1988; Wilkinson et al., 1997), making it an extremely useful tool (Binder, 2008, Serek et al., 2006a). It has been used successfully in a range of ornamental species to delay floral senescence including *Petunia* (Clevenger et al., 2004; Clark et al., 1999a; Gubrium et al., 2000; Wilkinson et al., 1997), *Dianthus* (Bovy et al., 1999), *Campanula* (Sriskandarajah et al., 2007) and *Kalanchoe* (Sanikhani et al., 2008). Other genes in the ethylene signalling pathway such as *EIN2*, which is down-stream of the receptor, have also been down-regulated in ornamental species such as Petunia (Shibuya et al., 2004) resulting in delayed senescence.

However, as discussed above, ethylene affects a wide range of developmental processes and physiological responses in the plant, thus a down-regulation of ethylene responses throughout the plant can have undesired effects such as root formation (Clark et al., 1999b), disease susceptibility (Shaw et al., 2002) and seed germination (Clevenger et al., 2004) which in turn affect production. Therefore this strategy is most effective when expression of the *etr1-1* mutant gene is driven by a flower specific promoter derived from e.g the Petunia MADS box gene CBM2 (Baudinette et al., 2000) or *fbp1* from *Petunia hybrida* (Raffeiner et al., 2009). This latter promoter was used successfully to delay senescence, and shown to be specific for buds, petals or stamens in transgenic *Dianthus, Campanula* and *Kalanchoe* (Bovy et al., 1999; Sanikhani et al., 2008; Sriskandarajah et al., 2007). Ethylene sensitivity to 1μl/l ethylene was completely abolished in kalanchoe (Sanikhani et al., 2008) and in both kalanchoe and campanula (Sriskandarajah et al., 2007) some lines were tolerant to levels of 2μl/l ethylene. Crucially plants were otherwise phenotypically normal in all three species.

Alternative pathways for reducing ethylene sensitivity have also been tested. A recent study by Christensen and Müller (2009) demonstrated that expression of *rol* genes can also enhance postharvest performance and increase ethylene tolerance in transgenic *Kalanchoe blossfeldiana,* even though the mechanisms involved are presently unknown. Possible mechanisms are via an alteration of hormone homeostasis and/or sugar metabolism and transport.

Although these approaches appear to be successful, there has been a lack of commercialisation in ornamentals and only very few transgenic lines have been commercialised (Chandler and Sanchez, 2012). One of the barriers is that while there are hundreds of ornamental cut flower species and thousands of varieties, only about fifty ornamental species are transformable (Chandler and Sanchez, 2012). A further barrier is ascribed to the cost and complexity of the regulatory process and lack of harmonisation of the regulations across different world markets. Furthermore, despite being the largest market for ornamentals, the European regulatory environment is one of the most stringent. Alternative strategies are also therefore still required.

1.2.2 Environmental strategies

In many situations, considerable ethylene emission occurs throughout the horticultural distribution chain, such as in producer or market refrigerators and storage chambers, inside packaging, and during transportation (Martínez-Romero et al., 2007). This ethylene comes from normal emission from plant organs or external sources, such as micro organism metabolism and pyrolysis of hydrocarbons in internal combustion engines (Cape, 2003; Chang and Bleecker, 2004).

A first key approach is to reduce exposure to exogenous ethylene e.g. by avoiding mixed loads of ethylene sensitive and producer species). However, exogenous and endogenous ethylene exert similar effects, thus, in order to avoid detrimental effects on cut flower quality, its detection and removal is advisable. Ethylene levels as low as 20 $\mu l\ l^{-1}$ (ppm) inside conservation chambers are enough to trigger unwanted ripening processes of climacteric fruits (Ivanov et al., 2005). In fact air concentrations higher than 0.100 $\mu l\ l^{-1}$ can accelerate ripening and senescence processes, inducing important loss of quality (Wills and Warton, 2000). This leads to a reduction in shelf-life, in a wide range of other commodities (Wills et al., 2001), as well as in cut flowers (Reid and Jiang, 2012). Consequently lower concentrations (0.100-0.015 $\mu l\ l^{-1}$) have been recommended in processing and storage areas (Wills and Warton, 2000).

To reduce ethylene levels, three main approaches can be taken: removal. oxidation or absorption often used in combination. Reduced temperature is also useful: in cut flowers which tolerate low temperature (Cevallos, and Reid. 2001) including snapdragon (Çelikel et al., 2010), rose (Çelikel and Reid, 2005) and Asteraceae such as gerbera and sunflower (Çelikel, and Reid. 2002), refrigerated storage is beneficial in conservation and transport, since ethylene production and sensitivity are greatly reduced at low temperatures. Temperatures of 0 to 1 °C (32 to 33.8 °F) and 95 to 99% RH are the recommended conditions for these cut flowers and forced air cooling is the common method for pre-cooling products prior to storage (Reid and Jiang, 2012).

Adequate ventilation of warehouses with fresh air has been classically used to remove ethylene for storing climacteric vegetables, fruits and cut flowers, however this procedure is not practicable in sealed environments (e.g. controlled atmosphere or some packaging formats) or where a precise control is required. Furthermore this method results in significant energy losses by increasing the temperature and lowering the humidity. Therefore, most commercial control systems have relied for a long time on both ventilation (often periodic) and ethylene adsorption/oxidation, using materials with suitable adsorption properties, in terms of pore structure (magnitude and distribution of pores) surface chemistry (type and quality of surface-bound functional groups), molecular sieving and oxidation capacity (Martínez-Romero et al., 2007).

Based on these mechanisms, a number of options are available commercially. These include membranes for filtration, small sachets inside the packages, enriched polyethylene films for modified atmosphere, including zeolites (Suslow, 1997; Limtrakul et al., 2001) and activated carbon (Choi et al., 2003; Bailén et al., 2006), as adsorbers. The efficiency of activated carbon as an adsorber is dependent on a wide range of physical and chemical properties as well as the material formulation, granular, powdered or fibre (Aygün et al., 2003). Martínez-Romero et al. (2007) found that the best results in terms of the rate of absorption of applied ethylene were obtained with granular (80%), followed by powered (70%) and fibre (40%) carbon. However, adsorption techniques on their own only transfer the ethylene to another phase (the solid adsorber matrix), rather than destroying it, and do not guarantee its total elimination.

Another strategy is oxidation. Inert matrices (e.g. alumina or silica gel) impregnated with potassium permanganate ($KMnO_4$), can be used as oxidising agents (Terry et al., 2007). However, performance of $KMnO_4$ depends on the percentage of active agent per matrix weight (usually 4 to 6%) and surface area of the substrate (Poças et al., 2008). In addition, in common with most of the ethylene scavengers, $KMnO_4$ has limited long-term efficacy in environments with high relative humidity (RH) (e.g. cold chambers, packaging, etc.) (Terry et al., 2007). Ozone (O_3) is an alternative gaseous oxidant, with good solubility in water and reactivity. Ozone acts as a powerful, residue-free ethylene oxidant and microbial disinfectant, which does not impair product appearance, texture, or scent. However it is highly unstable and decomposes easily into O_2 (Dickson et al., 1992). Furthermore, even though it has been listed as a GRAS (generally recognized as safe) material by the US Food and Drug Administration (FDA), its application is strictly regulated (Mahapatra et al., 2005).

The combination of an adsorbent with an oxidizer or catalyst (chemi-adsorption) enhances the efficacy of the two single strategies. Indeed, the use of some catalysts (palladium Pd, titanium Ti, copper Cu, rhodium Rh and cobalt Co) have also been shown to be effective in ethylene removal, by oxidising it to CO_2 and H_2O, even at low temperature and high RH (Conte et al., 1992; Maneerat et al., 2003). For example, results obtained by combining activated carbon with Pd have been far superior to $KMnO_4$-based scavengers at room temperature (20 °C) (Bailén et al., 2007; Terry et al., 2007). Pd fixed on activated carbon increased the efficiency of ethylene adsorbtion compared to activated carbon alone, even at low concentration (1% in weight), making this strategy sustainable for practical applications in common packaging and modified atmosphere packaging (MAP), despite the high cost of Pd (Martínez-Romero et al., 2007). However, this kind of system has several disadvantages, including the large quantity of adsorbent + catalyst required (due to adsorption of other environmental gases and the subsequent loss of efficacy over time), the requirement to reposition the material, and non-continuous operation (since regeneration of the adsorbent is necessary) (Martínez-Romero et al., 2009).

A refinement to the adsorbent + catalyst strategy that can be used to remove ethylene continuously has been developed based on activated carbon-1% Pd and the application of short heat pulses (Martínez-Romero et al., 2009). This system allows an increase in the rate of ethylene adsorbtion and oxidation (96-99% at 150-200 °C) and the elimination of deposits of other gases on the activated carbon, avoiding system saturation (auto-regeneration). It thus compares favourably to other non heated adsorbent-catalyst systems, with low CO_2 accumulation and without affecting the temperature of the storage environment. Silver (Ag) ions also appear attractive as a catalyst, because of their photoactivity, photocatalysis, and antibacterial activity (Verykios et al., 1980).

In summary, ventilation and air temperature control are commonly used during postharvest storage and transport of most cut flowers, together with adsorbers or oxidizers, while “ozonators” and catalytic degradation reactors are less widely used. However, recent advances in technology promise to expand the use of catalytic degradation in ethylene control in the floriculture industry (e.g. the carbon-heat hybrid ethylene scrubber; Martínez-Romero et al., 2009).

1.2.3 Chemical strategies

Use of ethylene biosynthesis inhibitors leads to a reduction in endogenous ethylene levels in the plant. These include cobalt ions (Lau and Yang 1976), aminooxyacetic acid (AOA) (Baker et al., 1982), aminoethoxyvinylglycine (AVG) (Baker et al., 1977; Wang et al., 1977), and methoxyvinylglycine (MVG) (Reid et al., 1992).

AVG and MVG are difficult to prepare and, thus, too expensive for practical use. Studies with AOA also indicated toxicological risks. Additionally, phytotoxicity is often a problem with these compounds. Therefore, new oxime ether derivatives of AOA have been recently proposed, including ethyl 4-[[2-[[(1-phenylmethylidene)amino]oxy]acetyl]oxy] butanoate was especially which is found to be more effective than AOA, (Zeng et al., 2012). However, these chemicals are only effective against the action of ethylene produced by the flower itself, and have no effect when flowers are exposed to exogenous ethylene, as can occur during transit and marketing. Therefore, their use is valuable for studies of ethylene biosynthesis, but they are unlikely to play an important role in horticultural practice.

More common treatments are the use of inhibitors of flower ethylene responses. For a vast number of ornamental species, blocking the plant’s response to ethylene via a chemical approach is an efficient strategy to enhance the longevity of the flowers (Serek et al. 2006a).

Ethylene action inhibitors interact with ethylene receptors and modulate ethylene responses. These include silver thiosulfate (STS) (Veen, 1979), 2,5-norbornadiene (2,5-NBD) (Sisler et al., 1983; Wang and Woodson, 1989), diazocyclopentadiene (DACP) (Blankenship and Sisler, 1993; Sisler et al., 1993; Serek et al., 1994) and 1-methylcyclopropene (1-MCP) (Serek et al., 1995b, 2006a). STS is a convenient ethylene inhibitor and has been widely used in commercial practice for a number of horticultural commodities (Veen, 1983). However, the use of silver raises environmental concerns, mainly related to disposal issues (Sisler et al., 1997; Marambio-Jones and Hock 2010). 2,5-NBD has a very disagreeable odour and requires continuous exposure to be effective, therefore it has very limited potential for commercial use (Sisler et al., 1990). Similarly, instability and explosive characteristics of DACP make it an unlikely candidate for commercial use (Serek et al., 2006b).

1-MCP was the first patented non-toxic ethylene action inhibitor (Sisler and Blankenship, 1996). 1-MCP treatment conditions and effects on floricultural crops have been reviewed by Blankenship and Dole (2003). Its high efficacy has been well documented in a range of ornamental species and it is now widely used commercially under the trade name of EthylBloc® and SmartFresh™ (Serek et al., 2006b). However, the gaseous nature of 1-MCP leads to difficulties with its use due to three key factors: (i) plant material must be kept in enclosed areas to prevent gas leakage, (ii) the effect of 1-MCP can be transitory in some plants, depending on the species, the concentrations, and lighting (Sisler et al., 1996a, b; Blankenship and Dole 2003; Kebenei et al., 2003; Feng et al., 2004; Apelbaum et al., 2008), thus some ornamentals require continuous or repeated applications, (Serek and Sisler, 2005; Serek et al., 2006b) and (iii) and the action of commercial formulations of 1-MCP appears to be strongly reduced by treatment temperature (0–5◦C) and by the presence of exogenous ethylene (Seglie et al., 2011a; Çelikel and Reid, 2002; Reid and Çelikel, 2008). Furthermore, many conventional 1-MCP delivery vehicles, such as cyclopropenes and cyclodextrins, have low preservative efficiency and, consequently, require high concentrations of active ingredients to be effective. These levels may induce side effects due to the high input levels (Sisler et al., 1996a, b, 1999). Advances have occurred to counter some of these limitations by developing 1-MCP-based compounds that can be applied in non-volatile formulations. Different cyclopropene salt compounds such as N,N-dipropyl(1-cyclopropenylmethyl)amine (DPCA) have been recently synthesized (Sisler et al., 2009) and used to protect several ornamentals against ethylene (Seglie et al., 2010). Cyclopropene salt compounds differ amongst each other in their chemical structure, but they all have a methyl group in the 1-position, onto which an amine is substituted. Such compounds can be used as a gas in a confined space or as a salt in open spaces. Moreover, recently, the company Floralife (Walterboro, SC) has released a novel treatment system 1-MCP sachets resembling tea bags. The bags are dipped in water just before being placed within a packed box; the water diffuses through the bag, and the 1-MCP in it is released into the air within the box. Preliminary experiments have shown this technique to be quite effective (Reid and Çelikel, 2008).

2. Nanotechnology for ethylene control

Nanotechnology can be defined as the design, characterization, production, and application of structures, devices, and systems by controlling the shape and size at the nanometer scale (Mousavi and Rezaei, 2011). Nanotechnology exploits the particular characteristics of nanoparticles (structures of 1 to 100 nm dimensions) and can be a very useful technology in a wide range of branches in science and industry. Understanding and controlling matter at the nanoscale interests researchers in the sciences, medicine, agriculture, and industry because a material's properties at the nanoscale can be very different from those at a larger scale (Yadollahi et al., 2010).

Nanotechnology is widely employed in the agriculture and food industry, with many applications at all stages of product production, processing, storing, packaging and transport (Mousavi and Rezaei, 2011). Uses of nanotechnology aim to increase production and decrease postharvest wastage. Nanoparticles and nanoporous materials can be used to carry ethylene action inhibitors, control growth and development of microorganisms and introduce a new generation of packaging coverage that controls gases and harmful UV rays while increasing strength, quality and packaging appearance (Yadollahi et al., 2010).

Application in the floriculture industry is still limited, nevertheless, a recent increase in nanotechnology research indicates a promising future for this technology throughout the supply chain (Figure 2). Recent results on the use of nanotechnology *sensu lato* for cut flower vase life improvement, focusing on ethylene control strategies, is discussed below.

2.1 Nanotechnology for ethylene detection and removal in the postharvest environment

2.1.1 Sensors using nanoparticles for the detection of ethylene

Ethylene gas sensors are used to detect and monitor the concentration of the gas in the environment. This can be aimed to prevent exposure of fruits and vegetables to detrimental levels of ethylene.

The most common nano-material used for detection in ethylene sensors is tin dioxide (stannic oxide, SnO_2) (Ivanov et al., 2005; Agarwal et al., 2012), others are tungsten trioxide (WO_3, Pitcher et al., 2003), palladium (Pd, Pietrucha and Lalevic, 1988), platinum (Pt, Winquist and Lundström, 1987), titanium dioxide (TiO_2, Zhang et al., 2002), and zinc oxide (ZnO, Kang et al., 2004).

In more sophisticated versions, WO_3-SnO_2 binary oxide, with uniform distribution of nano-WO_3 within a SnO_2 particle-based material, has been developed successfully (Pimtong-Ngam et al., 2007). Similarly, nano-Au/Co_3O_4, with gold catalyst nanoparticles dispersed on a nano-Co_3O_4 support surface, showed great potential, particularly for indoor environmental control of ethylene traces (Li et al., 2008). Most of these materials are used in resistor-based devices, where their conductivity increases or decreases as an effect of the exposure to different ethylene concentrations.

The usual techniques used to construct the sensing layer (e.g. ceramic paste, thick film printing, sol gel) require high-temperature heating and complex material mixing techniques. Furthermore, ethylene detection also requires expensive and complex methods such as quantum-cascade laser (Weidmann et al., 2004), gas chromatography (Butrym and Hartman, 1998), photoluminescence (Burstyn et al., 2005), and chemiluminescence (Nelson et al., 2000). Moreover, since metal oxide sensors are responsive to a wide spectrum of toxic and combustible gases, their selectivity needs to be improved. In this respect, multi-sensor arrays, including different metal oxides as sensing elements with partially overlapping sensitivities, as well as a modulated working temperature of the sensor, which alters the kinetics of adsorption and reaction at the sensor surface, allow significant improvements to the problem of selectivity (Ivanov et al., 2005). However, the problem of measuring ethylene levels continuously during storage of climacteric fruits or other fresh produce is critical because ethylene detectors are bulky and expensive (Agarwal et al., 2012; Cristescu et al., 2012).

A reversible chemioresistive sensor able to detect with high selectivity sub-ppm concentrations of ethylene and simply to be prepared from commercially available materials, has been recently proposed by Birgit et al. (2012).

Gas sensors containing nanostructures such as nanowires, e.g. the electronic detectors called electronic nose or e-nose, identify the odorant mimicking natural olfaction and estimate its concentration (Gardner and Bartlett, 1999). Sensors based on e-nose technology allow detection of the presence of ethylene in food products, because of contamination or spoilage (Valdés et al., 2009). Information from e-noses on fruit physiological states, based on changes in released volatiles, can be applied to retard the ripening process through exposure of the fruit to inhibitors (such as cyclopropene compounds as ethylene-receptor blockers) at the appropriate time, adjustments in storage conditions to preclude ethylene accumulation, and removal of bruised or damaged fruits, over-producing ethylene (Wilson and Baietto, 2009).

Nanomaterial-based sensors are widely applied in post harvest management of fruits (e.g. climacteric fruits like apples and peaches) and in the food industry (e.g. packaging of vegetables) (Cristescu et al., 2012). Nanosensors could therefore also help to prolong vase life of cut flowers, by enabling monitoring of ethylene concentrations in storage rooms of large growers and wholesale markets. However, a cost-benefit analysis is necessary to evaluate if this extra cost would be compensated by the extension of cut flower vase life in the different flower species and the specific market context. In addition, it has to be taken into account that monitoring ethylene levels in the supply chain would be useful only if the integrated ethylene exposure can be calculated and suitable data on the specific sensitivity of flowers to different levels of ethylene are available. In this respect, further research is needed in order to clarify the mechanisms of response to ethylene in the different plant species (reaction to a threshold value or an integral amount of ethylene). Furthermore, differences in sensitivity between species and even between varieties means that a very sophisticated system would be required which may not ultimately be cost-effective and may have limited applicability with mixed batches.

2.1.2 Nanocomposites and nanocatalyst for ethylene removal and photodegradation

Loss of quality and freshness of plant products during the time required for commercialization and consumption can be contained by means of the right selection of materials and packaging technologies, able to maintain the desired atmosphere. In this respect, nanotechnology can provide effective scavengers with selective ability to remove different gases (e.g. oxygen, ethylene). In particular, inclusion of nano-scale fillers (e.g. Pd) within the matrix can make plastic films more impermeable to ethylene (Neethirajan and Jayas, 2011). These nano-components help to create active packaging for fruits and vegetables, such as ethylene-scavenging bags, exhibiting barrier properties (Robinson and Morrison, 2010), or novel systems including nanoparticle-promoted absorbent matrices, such as Pd-enriched zeolite (Smith el al.,

2009) to include in classical packaging. Nanoparticulates work as small physical barriers to the movement of gas molecules, by obstructing the path of the gas through the material. Furthermore, they have a relatively larger surface area than larger fillers, which favours filler-matrix interactions and the performance of the composite, acting as nano-reinforcements. However, achieving optimal barrier and mechanical performance requires the correct concentration and an excellent dispersion of the nanoparticulates throughout the matrix.

The use of nano-fillers in polymer composites (mixtures of polymers with inorganic or organic additives) is leading to the development of polymer nanocomposites, which represent a radical alternative to conventional materials and offer extra benefits such as low density, transparency, good flow, better surface properties and recyclability (Sinha Ray and Okamoto, 2003).

The application of nanocomposites promises to expand the use of edible and biodegradable films for food packaging (Sinha Ray and Bousmina, 2005), which was strongly limited in the beginning because of the poor barrier properties and weak mechanical properties of natural polymers (Petersen et al., 1999). However, nowadays blending with other synthetic polymers or, less frequently, chemical adjustment allow their application to more severe circumstances (Rhim el al., 2013).

Some alternatives to active packaging (e.g. catalytic degradation) look very attractive as tools for ethylene control but they require expensive materials or techniques and still show a low cost effectiveness. Nano-catalytic degradation of ethylene, and other hazardous materials, is one of the most desirable and challenging goals in the development of environmentally friendly catalysts (Rickerby et al., 2000). It involves the actual destruction of organic contaminants rather than just the transfer from one phase to another. For practical ethylene removal, the best tested catalysts have been Pd and TiO_2 fixed on activated carbon (Rodríguez-Reinoso, 1997).

Titanium dioxide (TiO_2) has been the focus for light-activated photocatalytic degradation under ultraviolet (UV) irradiation, either from natural (sun) or artificial (lamps), because of its physical and chemical stability, low cost, availability and non-toxicity (Hussain et al., 2011). TiO_2 action is unaffected by relative humidity and is efficient at room temperature, however the constant need for UV light represents a limiting factor.

Silver (Ag) ions also show photoactivity, semiconductor photocatalysis, and antibacterial activity: nano-Ag absorbs and decomposes ethylene and can have more effective antibacterial activity than Ag (Hu and Fu, 2003). Thus, packaging films incorporating nano- Ag or TiO_2 (e.g. nanocomposite polyethylene film) contribute to preserve quality of fruits and vegetables, retarding senescence and decreasing microbial growth.

Application of nanocomposites and nanocatalysts in floriculture is still limited, however current advances in packaging materials and formats (reviewed by Rhim et al., 2013) and successful tests on photocatalytic reactor prototypes (Hussain et al., 2011; Li et al., 2008) demonstrate how these technologies are potentially economically viable for commercial application to cut flowers (Figure 2).

2.2 Nanoparticles and nanoporous materials for ethylene action inhibition

Recent advances in nanotechnology demonstrate the increased attention that is now being paid to the supramolecular assembly of simple components. The design of new biomaterials based on nanoscale structural characteristics can be expected to provide many potential applications. Nano-sized colloidal carriers have recently been developed and proposed for drug delivery, since their use can solubilise poorly water-soluble active principles and provide prolonged release, as well as improving their bioavailability and in some cases modifying the kinetic parameters (Cavalli et al., 2006). They can also protect active components from degradation. Among colloidal carriers, nanoparticles have in particular been described as a new technological approach (Cavalli et al., 2006).

Nanometer-sized silver (Ag^+) particles (NS) are used in various applications as anti-microbials (Furno et al., 2004). NS have a high surface area to volume ratio and because of this property, they are considered to be more effective at preventing growth of bacteria and other microorganisms than the components of oxidation states of Ag (Furno et al., 2004). NS release Ag^+ (Lok et al., 2007), which has been reported to interact with cytoplasmic components and nucleic acids, to inhibit respiratory chain enzymes and to interfere with membrane permeability (Russell and Hugo, 1994; Park et al., 2005). Use of NS is becoming increasingly widespread in medicine, fabrics, water purification and various other industrial and non-plant applications (Jain and

Pradeep, 2005; Dubas et al., 2006 and Chen and Schluesener, 2008). Their use as a pulse and vase solution treatment for cut flowers is relatively new. Studies have investigated the effectiveness of NS in extending the vase life of some cut flowers, including carnations, gerberas, acacias, and roses (Liu et al., 2009; Solgi et al., 2009; Lü et al., 2010; Liavali and Zarchini, 2012; Liu et al., 2012, Moradi et al., 2012, and Nazemi and Ramezanian, 2013). The positive effect of a NS pulse treatment was attributed to inhibition of bacterial growth in the vase solution and at the cut stem ends. However, physiological activity of Ag^+ from NS is also a possibility. As with other cations (e.g. K^+, Ca^{2+}), Ag^+ can have positive effects on plant stem hydraulic conductivity (van Ieperen, 2007). Also, Ag^+ is considered to be a general inhibitor of aquaporins (Niemietz and Tyerman, 2002), improving water relations (Lü et al., 2010). Besides antibacterial and acidic effects, NS could act as antiethylene agents. Ag+, generally applied as STS, is an effective ethylene action inhibitor (Beyer, 1976; Veen, 1979). Kim et al. (2005) suggested that NS acted as anti-ethylene agents on cut Asiatic hybrid *Lilium* ‘Dream Land’ and Oriental hybrid *Lilium* ‘Sibera’ (Lü et al., 2010).

Cyclodextrins (CDs) are nanometric biomaterials synthesised by enzymatic action on hydrolysed starch. They have a characteristic toroidal shape, which forms a well-defined truncated cone-shaped lipophilic cavity. CDs are able to include compounds whose geometry and polarity are compatible with that of their cavity. Furthermore, chemical modifications of CDs have been studied in an attempt to form inclusion complexes with hydrophilic or high-molecular-weight drugs too (Trotta et al., 2012).

One approach is to synthesize cross-linked CD-based polymers in order to prepare insoluble multifunctional CD derivatives. These polymers can be obtained by reacting native CDs with a cross-linking agent that, after reaction, exerts its own properties and influences the behaviour of the CD unit. Although insoluble cross-linked CD polymers were first reported a long time ago, the term cyclodextrin nanosponges (CD-NSs) was first used by Li and Ma (1998) to indicate a cross-linked β-CD with organic diisocyanates leading to an insoluble network that showed a very high inclusion constant with several organic pollutants. Generally speaking, CD-NSs are hyper-cross-linked CDs that can be obtained with α, β and γ CDs, either alone or as mixtures containing relevant amounts of linear dextrin, cross-linked with a suitable cross-linking agent. CD-NSs were initially used for removing persistent organic pollutants (POPs) in water purification (Li and Ma, 1999; Arkas et al., 2006). Then, further studies were carried out in the preparation of cosmetics. Lately, medical and pharmaceutical applications have been of particular relevance, in which CD-NSs are used as carries for drug delivery (Trotta et al., 2012; Trotta, 2011).

Currently, evaluation of the potential for the use of CD-NSs in the field of agriculture appears an important research goal. CD-NSs hold a promising future in various applications such as enhanced product performance, improved thermal, physical, and chemical stability, and extended release and bioavailability.

In the postharvest context, CD-NSs (patented by Trotta et al., 2006) have been proposed as a delivery system capable of slowing the release of 1-MCP (Devecchi et al., 2009). These have the benefits of requiring reduced active ingredient dosages and reduced number of delivery times, as compared to the gaseous commercial product. In carnation, the inclusion of 1-MCP in a β-CD-NS structure has been shown to be effective not only in prolonging cut flower vase life (5 days more than gaseous 1-MCP; Seglie et al., 2011a; Seglie et al., 2011b) but also in controlling *Botrytis cinerea* damage (a 16% reduction in the development of grey mould; Seglie et al., 2012). The superior efficacy in improving postharvest perfomances of 1-MCP included in β-CD-NS has been seen also in a number of other ethylene sensitive species (*Anemone coronaria* L. multicolor*, Ranunculus asiaticus* L. 'Minou Abrown'*, Helianthus annuus* L. 'SunrichOrange'*, Rosa hybrida* L. 'Jupiter'*, Paeonia lactiflora* Pall. 'Sarah Bernhardt'*,* and *Papaver nudicaule* L. multicolor.) (Seglie et al., 2013). 1-MCP is a highly unstable and reactive gas that very quickly dimerizes even at room temperature. This dimer has no anti-ethylene activity. Most likely β-CD-NS stabilizes the included 1-MCP thus preserving its properties.

Therefore, 1-MCP included in β-CD-NS may be a promising user-friendly formulation, with low environmental impact, for prolonging the shelf life and controlling fungal diseases of cut flowers in the postharvest environment, although the mechanism of action needs further elucidation (Seglie et al., 2013). This new formulation appears moreover to have important economic implications: its application does not require an air-tight environment, allowing easier and faster open-space application, a major advantage for field production in ornamental nurseries/gardens. However, future commercial use of 1-MCP included in β-CD-NS will require more development to optimize chemical concentration and to evaluate this compound on an extended number of plant species in a range of environments.

3. Conclusions and Future prospects

Although a range of solutions exist currently to reduce the impact of ethylene on postharvest floral longevity through the supply chain, none currently meets all the requirements. However, recent progress in the development of nanotechnological strategies suggests that they have a lot to offer. Nanotechnologies could help to overcome postharvest quality and safety issues by developing user friendly green tools. Nano-scale systems could be applied to cut flowers for ethylene detection in the store environment (nano-metals based sensors) and along the distribution chain (nano-chip labels). They could also be used for ethylene removal (nano-metals for photocatalitic degradation in the warehouse or nanocomposites for scrubbing in active packaging). The use of new natural formulations (e.g. nanosponges) able to increase the bio-availability of the active ingredients has already been shown to enable a reduction in commonly applied concentrations of agrochemicals, helping to minimize the impact of agriculture on the environment and to reduce production costs. However, the efficiency and the economic benefit of applying each strategy to the flower industry needs to be evaluated in the different crop/market contexts.

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Figure 1 – Schematic view of ethylene control strategies in production and distribution chain of ethylene-sensitive plant species.

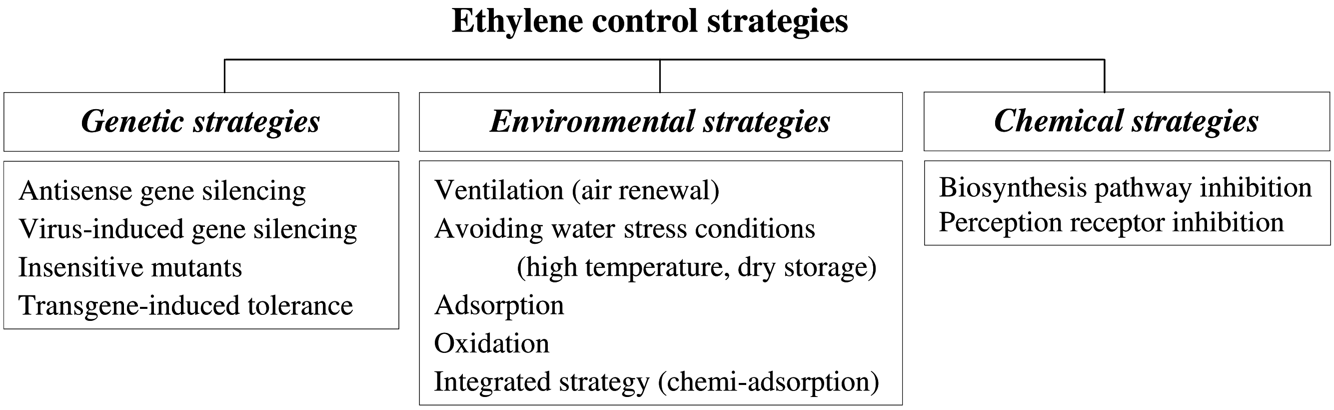

Figure 2 – Example of futuristic nanotechnology-based system for ethylene control in ethylene-sensitive cut flowers.