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Ethylene control in cut flowers: Classical and innovative approaches

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1	Ethylene control in cut flowers: classical and innovative approaches
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13	
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17	

18 Highlights

- 19 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
- 22 Nanocatalysts to promote ethylene catalytic degradation in the warehouse
- Nanoparticles and nanosponges as carriers of drugs for ethylene action inhibition
- 24

25 Abstract

Ethylene-mediated premature floral senescence and petal or flower abscission affect 26 postharvest longevity of several species used as cut flowers. Exposure to exogenous 27 or endogenously produced ethylene can be controlled in several ways. These include 28 the use of ethylene biosynthesis inhibitors or ethylene action inhibitors, and ethylene 29 removal technologies. In addition, genetic modification can be very effective in 30 controlling ethylene synthesis and perception. We review here the potential for 31 applications of nanotechnology to control ethylene levels and postharvest 32 management in the flower industry. Already nanosponges have been shown to 33

enhance efficacy of the ethylene inhibitor, 1-MCP, in several flower species. In 34 carnation, 1-MCP included in nanosponges also allowed better control of Botrytis 35 cinerea damage. However other applications are also considered based on 36 successes in the use of this technology to increase agricultural production and 37 decrease postharvest waste. Nano-metal based sensors could be used for detection 38 of ethylene in the store and to label the product along the distribution chain. 39 Furthermore, nanocomposites could be included as scavengers for ethylene removal 40 in active packaging, and nanocatalysts could promote ethylene catalytic degradation 41 in the warehouse. Nanoparticles could also be introduced into a new generation of 42 packaging to control effects of gases and UV, and increase strength, quality and 43 packaging appearance. This review highlights recent results on the use of 44 nanotechnology sensu lato and potential application for cut flower vase life 45 improvement, focusing on ethylene control strategies. 46

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68 **1. Introduction**

Postharvest performance is a key factor in the commercial value of cut flowers. 69 Although external quality criteria such as appearance, colour and uniformity, are the 70 major variables that influence the consumer's decision to purchase cut flowers, their 71 longevity is fundamental to convince the consumer to re-purchase them (Reid and 72 Jiang, 2012). As a fresh commodity and because of their extreme sensitivity, cut 73 74 flowers are vulnerable to large postharvest losses. In addition to developmental senescence, cut flowers are also subject to leaf discoloration, premature wilting, and 75 disease from moulds and fungal pathogens, An integrated approach is therefore 76 adopted to maintain quality throughout the distribution chain to reduce water loss 77 (e.g. avoiding high temperatures), control disease (such as *Botrytis* and *Alternaria*) 78 and to limit cut flower ageing (avoiding prolonged cold storage). 79

Advances in postharvest science and technology aim to provide information for the 80 horticultural industry to enable them to supply attractive and long-lived flowers to 81 consumers. Indeed in the last ten years substantial progress in postharvest 82 technologies has been achieved including novel packaging, storage and transport 83 systems, pest and disease control for market access, senescence control, supply 84 chain optimization, and track and trace systems to ensure delivery of premium quality 85 products to markets (Toivonen, 2007; Michailides and Manganaris, 2009; Sharma, 86 2010). Chemicals are used extensively in modern agriculture in order to improve 87 yield and quality. However, their use poses environmental and public health 88 concerns. Many chemicals that affect ethylene synthesis or its action, which are 89 currently in use to extend the shelf life of flowers, may be soon banned due to their 90 environmental impact. Over the last decades, environmentally and health-friendly 91 production methods and conscientious use of resources have become crucial for 92 reaching the goal of more sustainable plant production. techniques and systems 93 need to be developed. Thus further progress will require an integration of available 94 bio-, info- and nano-technologies through a systems biology approach. 95

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1.1 Role of ethylene in floral senescence

⁹⁹ Ethylene is a simple molecule composed of two carbon atoms symmetrically linked to ¹⁰⁰ by a double bond and it naturally occurs in gaseous form. It is, furthermore, a plant ¹⁰¹ growth regulator involved in the regulation of a wide range of different physiological ¹⁰² processes, including germination, growth, floral initiation and opening, both leaf and ¹⁰³ floral senescence as well as organ abscission and fruit ripening (Yoo et al., 2009).

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105 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 μ l 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. 113 Ethylene is produced by many plant tissues (Gane, 1934) and other sources, 114 including bacterial and fungal fermentation processes, and pyrolysis of hydrocarbons, 115 which releases ethylene as a component of air pollutants (Cape, 2003), all of which 116 can thus affect the longevity of cut flowers in the horticultural supply chain. Ethylene 117 is biologically active at very low concentrations (nl-µl l⁻¹), but there are significant 118 differences in ethylene sensitivity between species and even cultivars of the same 119 species (Serek et al., 2006b; Scariot et al., 2008). A detailed classification of flowers 120 based on ethylene sensitiveness is reported by van Doorn (2001). 121

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123 1.1.2 Plant species: sensitivity and effects

Responses to ethylene vary widely according to the species (Reid and Wu, 1992) 124 although they are often consistent within either families or subfamilies (van Doorn, 125 2001). Ethylene-sensitive species include a number of important cut flowers. For 126 example petals of orchids (Phalaenopsis), Hibiscus (Celikel and Reid, 2002), and 127 carnation (Diathus caryophyllus) (Serek et al., 1995a,b) wilt in response to ethylene. 128 In other species, such as Antirrhinum majus, Rosa hybrida (Serek et al., 1995a), and 129 wax flower (Chamelaucium uncinatum) (Macnish et al., 2000), ethylene induces petal 130 or flower abscission. 131

Ethylene sensitive flowers can be classified into three types (Kumar et al., 2008). 132 First, those like carnation and petunia where senescence is regulated by an 133 increased amount of ethylene production either with ageing or following pollination 134 (Serek et al., 1995a). Second, like cyclamen, which only become sensitive to 135 ethylene and produce increased amounts of the hormone when they are pollinated 136 (Halevy et al., 1984). Third, like rose, which are sensitive to ethylene upon flower bud 137 opening but do not produce elevated amounts of ethylene as they age (Kumar et al., 138 2008). 139

As well as accelerating petal senescence and deterioration, ethylene (either 140 endogenous or from an external source) can induce other undesirable physiological 141 disorders to vegetative and flowering organs during postharvest storage of cut 142 flowers both in monocotyledons and dicotyledons including pathogen susceptibility 143 (McKenzie and Lovell, 1992; van Doorn, 2001). For example, Botrytis cinerea is one 144 of the most significant postharvest fungal pathogens causing losses in ornamental 145 plants. Disease caused by this fungus has been shown to be enhanced by the 146 presence of ethylene in rose and carnation (Elad, 1988; Seglie et al., 2012). 147 However, depending on the type of pathogen and plant species, the role of ethylene 148 can be dramatically different. Indeed plants deficient in ethylene signaling may show 149 either increased susceptibility or increased resistance (Elad, 1988). 150

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.

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157 1.2 Ethylene control strategies

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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).

171

172 1.2.1 Genetic strategies

173 Changes in gene expression during petal senescence have been studied through 174 transcriptomics of a number of model flowers (e.g., *Petunia*, *Arabidopsis*) and cut 175 flower species (e.g., *Alstroemeria*, *Dianthus*, *Iris*, *Sandersonia*) (Rogers, 2013). In 176 species where petal senescence is ethylene-sensitive groups of genes can be 177 identified that are ethylene regulated, comprising transcription factors, genes 178 encoding for enzymes in the biosynthetic pathway for ethylene production, ethylene 179 receptors and ethylene signalling and responsive genes (Rogers 2013).

Ethylene biosynthesis is primarily regulated by 1-aminocyclopropane-1-carboxylic 180 acid (ACC) synthase (ACS) and ACC oxidase (ACO) and an early success by the 181 company Florigene in delaying carnation floral senescence was through antisense 182 down-regulation of ACO (Savin et al., 1995). This success was closely followed by 183 down-regulation of ACO in other flower species such as begonia (Einset and 184 Kopperud, 1995) and torenia (Aida et al, 1998). Down-regulation of the ACS gene in 185 carnation also reduced ethylene production (Kiss et al., 2000). Use of antisense 186 sequences in Petunia for ACO and ACS, derived heterologously from broccoli also 187 delayed floral senescence (Huang et al., 2007) showing that the approach can be 188 used more broadly. However, these strategies have no effect when flowers are 189 exposed to exogenous ethylene, as can occur during transit and marketing. 190

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 197 et al., 2006a). It has been used successfully in a range of ornamental species to 198 delay floral senescence including *Petunia* (Clevenger et al., 2004; Clark et al., 1999a; 199 Gubrium et al., 2000; Wilkinson et al., 1997), Dianthus (Bovy et al., 1999), 200 Campanula (Sriskandarajah et al., 2007) and Kalanchoe (Sanikhani et al., 2008). 201 Other genes in the ethylene signalling pathway such as *EIN2*, which is down-stream 202 of the receptor, have also been down-regulated in ornamental species such as 203 Petunia (Shibuya et al., 2004) resulting in delayed senescence. 204

However, as discussed above, ethylene affects a wide range of developmental 205 processes and physiological responses in the plant, thus a down-regulation of 206 ethylene responses throughout the plant can have undesired effects such as root 207 formation (Clark et al., 1999b), disease susceptibility (Shaw et al., 2002) and seed 208 germination (Clevenger et al., 2004) which in turn affect production. Therefore this 209 strategy is most effective when expression of the *etr1-1* mutant gene is driven by a 210 flower specific promoter derived from e.g the Petunia MADS box gene CBM2 211 (Baudinette et al., 2000) or fbp1 from Petunia hybrida (Raffeiner et al., 2009). This 212 latter promoter was used successfully to delay senescence, and shown to be specific 213 for buds, petals or stamens in transgenic *Dianthus, Campanula* and *Kalanchoe* (Bovy 214 et al., 1999; Sanikhani et al., 2008; Sriskandarajah et al., 2007). Ethylene sensitivity 215 to 1µl/l ethylene was completely abolished in kalanchoe (Sanikhani et al., 2008) and 216 in both kalanchoe and campanula (Sriskandarajah et al., 2007) some lines were 217 tolerant to levels of 2µl/l ethylene. Crucially plants were otherwise phenotypically 218 normal in all three species. Alternative pathways for reducing ethylene sensitivity 219 have also been tested. A recent study by Christensen and Müller (2009) 220 demonstrated that expression of rol genes can also enhance postharvest 221 performance and increase ethylene tolerance in transgenic Kalanchoe blossfeldiana, 222 even though the mechanisms involved are presently unknown. Possible mechanisms 223 are via an alteration of hormone homeostasis and/or sugar metabolism and transport. 224 Although these approaches appear to be successful, there has been a lack of 225 commercialisation in ornamentals and only very few transgenic lines have been 226 commercialised (Chandler and Sanchez, 2012). One of the barriers is that while 227 there are hundreds of ornamental cut flower species and thousands of varieties, only 228 about fifty ornamental species are transformable (Chandler and Sanchez, 2012). A 229

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.

235

236 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 243 mixed loads of ethylene sensitive and producer species). However, exogenous and 244 endogenous ethylene exert similar effects, thus, in order to avoid detrimental effects 245 on cut flower quality, its detection and removal is advisable. Ethylene levels as low as 246 20 μ l l⁻¹ (ppm) inside conservation chambers are enough to trigger unwanted 247 ripening processes of climacteric fruits (Ivanov et al., 2005). In fact air concentrations 248 higher than 0.100 µl l⁻¹ can accelerate ripening and senescence processes, inducing 249 important loss of quality (Wills and Warton, 2000). This leads to a reduction in shelf-250 life, in a wide range of other commodities (Wills et al., 2001), as well as in cut flowers 251 (Reid and Jiang, 2012). Consequently lower concentrations (0.100-0.015 μ l l⁻¹) have 252 been recommended in processing and storage areas (Wills and Warton, 2000). 253

To reduce ethylene levels, three main approaches can be taken: removal. oxidation 254 or absorption often used in combination. Reduced temperature is also useful: in cut 255 flowers which tolerate low temperature (Cevallos, and Reid. 2001) including 256 snapdragon (Celikel et al., 2010), rose (Celikel and Reid, 2005) and Asteraceae such 257 as gerbera and sunflower (Celikel, and Reid. 2002), refrigerated storage is beneficial 258 in conservation and transport, since ethylene production and sensitivity are greatly 259 reduced at low temperatures. Temperatures of 0 to 1 °C (32 to 33.8 °F) and 95 to 260 99% RH are the recommended conditions for these cut flowers and forced air cooling 261 is the common method for pre-cooling products prior to storage (Reid and Jiang, 262

263 2012).

Adequate ventilation of warehouses with fresh air has been classically used to 264 remove ethylene for storing climacteric vegetables, fruits and cut flowers, however 265 this procedure is not practicable in sealed environments (e.g. controlled atmosphere 266 or some packaging formats) or where a precise control is required. Furthermore this 267 method results in significant energy losses by increasing the temperature and 268 lowering the humidity. Therefore, most commercial control systems have relied for a 269 long time on both ventilation (often periodic) and ethylene adsorption/oxidation, using 270 materials with suitable adsorption properties, in terms of pore structure (magnitude 271 and distribution of pores) surface chemistry (type and quality of surface-bound 272 functional groups), molecular sieving and oxidation capacity (Martínez-Romero et al., 273 2007). 274

Based on these mechanisms, a number of options are available commercially. These 275 include membranes for filtration, small sachets inside the packages, enriched 276 polyethylene films for modified atmosphere, including zeolites (Suslow, 1997; 277 Limtrakul et al., 2001) and activated carbon (Choi et al., 2003; Bailén et al., 2006), as 278 adsorbers. The efficiency of activated carbon as an adsorber is dependent on a wide 279 range of physical and chemical properties as well as the material formulation, 280 granular, powdered or fibre (Aygün et al., 2003). Martínez-Romero et al. (2007) found 281 that the best results in terms of the rate of absorption of applied ethylene were 282 obtained with granular (80%), followed by powered (70%) and fibre (40%) carbon. 283 However, adsorption techniques on their own only transfer the ethylene to another 284 phase (the solid adsorber matrix), rather than destroying it, and do not guarantee its 285 total elimination. 286

Another strategy is oxidation. Inert matrices (e.g. alumina or silica gel) impregnated 287 with potassium permanganate (KMnO₄), can be used as oxidising agents (Terry et 288 al., 2007). However, performance of KMnO₄ depends on the percentage of active 289 agent per matrix weight (usually 4 to 6%) and surface area of the substrate (Poças et 290 al., 2008). In addition, in common with most of the ethylene scavengers, KMnO₄ has 291 limited long-term efficacy in environments with high relative humidity (RH) (e.g. cold 292 chambers, packaging, etc.) (Terry et al., 2007). Ozone (O_3) is an alternative gaseous 293 oxidant, with good solubility in water and reactivity. Ozone acts as a powerful, 294 residue-free ethylene oxidant and microbial disinfectant, which does not impair 295

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) 300 enhances the efficacy of the two single strategies. Indeed, the use of some catalysts 301 (palladium Pd, titanium Ti, copper Cu, rhodium Rh and cobalt Co) have also been 302 shown to be effective in ethylene removal, by oxidising it to CO₂ and H₂O, even at 303 low temperature and high RH (Conte et al., 1992; Maneerat et al., 2003). For 304 example, results obtained by combining activated carbon with Pd have been far 305 superior to KMnO₄-based scavengers at room temperature (20 °C) (Bailén et al., 306 2007; Terry et al., 2007). Pd fixed on activated carbon increased the efficiency of 307 ethylene adsorbtion compared to activated carbon alone, even at low concentration 308 (1% in weight), making this strategy sustainable for practical applications in common 309 packaging and modified atmosphere packaging (MAP), despite the high cost of Pd 310 (Martínez-Romero et al., 2007). However, this kind of system has several 311 disadvantages, including the large quantity of adsorbent + catalyst required (due to 312 adsorption of other environmental gases and the subsequent loss of efficacy over 313 time), the requirement to reposition the material, and non-continuous operation (since 314 regeneration of the adsorbent is necessary) (Martínez-Romero et al., 2009). 315

A refinement to the adsorbent + catalyst strategy that can be used to remove 316 ethylene continuously has been developed based on activated carbon-1% Pd and 317 the application of short heat pulses (Martínez-Romero et al., 2009). This system 318 allows an increase in the rate of ethylene adsorbtion and oxidation (96-99% at 150-319 200 °C) and the elimination of deposits of other gases on the activated carbon, 320 avoiding system saturation (auto-regeneration). It thus compares favourably to other 321 non heated adsorbent-catalyst systems, with low CO₂ accumulation and without 322 affecting the temperature of the storage environment. Silver (Ag) ions also appear 323 attractive as a catalyst, because of their photoactivity, photocatalysis, and 324 antibacterial activity (Verykios et al., 1980). 325

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).

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333 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. 338 Studies with AOA also indicated toxicological risks. Additionally, phytotoxicity is often 339 a problem with these compounds. Therefore, new oxime ether derivatives of AOA 340 have been recently proposed, including ethyl 4-[[2-[[(1-341 phenylmethylidene)amino]oxy]acetyl]oxy] butanoate was especially which is found to 342 be more effective than AOA, (Zeng et al., 2012). However, these chemicals are only 343 effective against the action of ethylene produced by the flower itself, and have no 344 effect when flowers are exposed to exogenous ethylene, as can occur during transit 345 and marketing. Therefore, their use is valuable for studies of ethylene biosynthesis, 346 but they are unlikely to play an important role in horticultural practice. 347

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 352 responses. These include silver thiosulfate (STS) (Veen, 1979), 2,5-norbornadiene 353 (2,5-NBD) (Sisler et al., 1983; Wang and Woodson, 1989), diazocyclopentadiene 354 (DACP) (Blankenship and Sisler, 1993; Sisler et al., 1993; Serek et al., 1994) and 1-355 methylcyclopropene (1-MCP) (Serek et al., 1995b, 2006a). STS is a convenient 356 ethylene inhibitor and has been widely used in commercial practice for a number of 357 horticultural commodities (Veen, 1983). However, the use of silver raises 358 environmental concerns, mainly related to disposal issues (Sisler et al., 1997; 359 Marambio-Jones and Hock 2010). 2,5-NBD has a very disagreeable odour and 360 requires continuous exposure to be effective, therefore it has very limited potential for 361

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 364 Blankenship, 1996). 1-MCP treatment conditions and effects on floricultural crops 365 have been reviewed by Blankenship and Dole (2003). Its high efficacy has been well 366 documented in a range of ornamental species and it is now widely used commercially 367 under the trade name of EthylBloc[®] and SmartFresh[™] (Serek et al., 2006b). 368 However, the gaseous nature of 1-MCP leads to difficulties with its use due to three 369 key factors: (i) plant material must be kept in enclosed areas to prevent gas leakage, 370 (ii) the effect of 1-MCP can be transitory in some plants, depending on the species, 371 the concentrations, and lighting (Sisler et al., 1996a, b; Blankenship and Dole 2003; 372 Kebenei et al., 2003; Feng et al., 2004; Apelbaum et al., 2008), thus some 373 ornamentals require continuous or repeated applications, (Serek and Sisler, 2005; 374 Serek et al., 2006b) and (iii) and the action of commercial formulations of 1-MCP 375 appears to be strongly reduced by treatment temperature (0-5°C) and by the 376 presence of exogenous ethylene (Seglie et al., 2011a; Celikel and Reid, 2002; Reid 377 and Çelikel, 2008). Furthermore, many conventional 1-MCP delivery vehicles, such 378 as cyclopropenes and cyclodextrins, have low preservative efficiency and, 379 consequently, require high concentrations of active ingredients to be effective. These 380 levels may induce side effects due to the high input levels (Sisler et al., 1996a, b, 381 1999). Advances have occurred to counter some of these limitations by developing 1-382 MCP-based compounds that can be applied in non-volatile formulations. Different 383 cyclopropene salt compounds such as N,N-dipropyl(1-cyclopropenylmethyl)amine 384 (DPCA) have been recently synthesized (Sisler et al., 2009) and used to protect 385 several ornamentals against ethylene (Seglie et al., 2010). Cyclopropene salt 386 compounds differ amongst each other in their chemical structure, but they all have a 387 methyl group in the 1-position, onto which an amine is substituted. Such compounds 388 can be used as a gas in a confined space or as a salt in open spaces. Moreover, 389 recently, the company Floralife (Walterboro, SC) has released a novel treatment 390 system 1-MCP sachets resembling tea bags. The bags are dipped in water just 391 before being placed within a packed box; the water diffuses through the bag, and the 392 1-MCP in it is released into the air within the box. Preliminary experiments have 393 shown this technique to be quite effective (Reid and Celikel, 2008). 394

395

2. Nanotechnology for ethylene control

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Nanotechnology can be defined as the design, characterization, production, and 398 application of structures, devices, and systems by controlling the shape and size at 399 the nanometer scale (Mousavi and Rezaei, 2011). Nanotechnology exploits the 400 particular characteristics of nanoparticles (structures of 1 to 100 nm dimensions) and 401 can be a very useful technology in a wide range of branches in science and industry. 402 Understanding and controlling matter at the nanoscale interests researchers in the 403 sciences, medicine, agriculture, and industry because a material's properties at the 404 nanoscale can be very different from those at a larger scale (Yadollahi et al., 2010). 405

Nanotechnology is widely employed in the agriculture and food industry, with many 406 applications at all stages of product production, processing, storing, packaging and 407 transport (Mousavi and Rezaei, 2011). Uses of nanotechnology aim to increase 408 production and decrease postharvest wastage. Nanoparticles and nanoporous 409 materials can be used to carry ethylene action inhibitors, control growth and 410 development of microorganisms and introduce a new generation of packaging 411 coverage that controls gases and harmful UV rays while increasing strength, quality 412 and packaging appearance (Yadollahi et al., 2010). 413

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.

419

420 2.1 Nanotechnology for ethylene detection and removal in the postharvest421 environment

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423 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, SnO₂) (Ivanov et al., 2005; Agarwal et al., 2012), others are tungsten trioxide (WO₃, Pitcher et al., 2003), palladium (Pd, Pietrucha and Lalevic, 1988), platinum (Pt, Winquist and Lundström, 1987), titanium dioxide (TiO₂, Zhang et al., 2002), and zinc oxide (ZnO, Kang et al., 2004).

In more sophisticated versions, WO₃-SnO₂ binary oxide, with uniform distribution of 432 nano-WO₃ within a SnO₂ particle-based material, has been developed successfully 433 (Pimtong-Ngam et al., 2007). Similarly, nano-Au/Co₃O₄, with gold catalyst 434 nanoparticles dispersed on a nano-Co₃O₄ support surface, showed great potential, 435 particularly for indoor environmental control of ethylene traces (Li et al., 2008). Most 436 of these materials are used in resistor-based devices, where their conductivity 437 increases or decreases as an effect of the exposure to different ethylene 438 concentrations. 439

The usual techniques used to construct the sensing layer (e.g. ceramic paste, thick 440 film printing, sol gel) require high-temperature heating and complex material mixing 441 techniques. Furthermore, ethylene detection also requires expensive and complex 442 methods such as quantum-cascade laser (Weidmann et al., 2004), gas 443 chromatography (Butrym and Hartman, 1998), photoluminescence (Burstyn et al., 444 2005), and chemiluminescence (Nelson et al., 2000). Moreover, since metal oxide 445 sensors are responsive to a wide spectrum of toxic and combustible gases, their 446 selectivity needs to be improved. In this respect, multi-sensor arrays, including 447 different metal oxides as sensing elements with partially overlapping sensitivities, as 448 well as a modulated working temperature of the sensor, which alters the kinetics of 449 adsorption and reaction at the sensor surface, allow significant improvements to the 450 problem of selectivity (Ivanov et al., 2005). However, the problem of measuring 451 ethylene levels continuously during storage of climacteric fruits or other fresh 452 produce is critical because ethylene detectors are bulky and expensive (Agarwal et 453 al., 2012; Cristescu et al., 2012). 454

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 460 on e-nose technology allow detection of the presence of ethylene in food products, 461 because of contamination or spoilage (Valdés et al., 2009). Information from e-noses 462 on fruit physiological states, based on changes in released volatiles, can be applied 463 to retard the ripening process through exposure of the fruit to inhibitors (such as 464 cyclopropene compounds as ethylene-receptor blockers) at the appropriate time, 465 adjustments in storage conditions to preclude ethylene accumulation, and removal of 466 bruised or damaged fruits, over-producing ethylene (Wilson and Baietto, 2009). 467

Nanomaterial-based sensors are widely applied in post harvest management of fruits 468 (e.g. climacteric fruits like apples and peaches) and in the food industry (e.g. 469 packaging of vegetables) (Cristescu et al., 2012). Nanosensors could therefore also 470 help to prolong vase life of cut flowers, by enabling monitoring of ethylene 471 concentrations in storage rooms of large growers and wholesale markets. However, a 472 cost-benefit analysis is necessary to evaluate if this extra cost would be 473 compensated by the extension of cut flower vase life in the different flower species 474 and the specific market context. In addition, it has to be taken into account that 475 monitoring ethylene levels in the supply chain would be useful only if the integrated 476 ethylene exposure can be calculated and suitable data on the specific sensitivity of 477 flowers to different levels of ethylene are available. In this respect, further research is 478 needed in order to clarify the mechanisms of response to ethylene in the different 479 plant species (reaction to a threshold value or an integral amount of ethylene). 480 Furthermore, differences in sensitivity between species and even between varieties 481 means that a very sophisticated system would be required which may not ultimately 482 be cost-effective and may have limited applicability with mixed batches. 483

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485 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, 493 exhibiting barrier properties (Robinson and Morrison, 2010), or novel systems 494 including nanoparticle-promoted absorbent matrices, such as Pd-enriched zeolite 495 (Smith el al., 2009) to include in classical packaging. Nanoparticulates work as small 496 physical barriers to the movement of gas molecules, by obstructing the path of the 497 gas through the material. Furthermore, they have a relatively larger surface area than 498 larger fillers, which favours filler-matrix interactions and the performance of the 499 composite, acting as nano-reinforcements. However, achieving optimal barrier and 500 mechanical performance requires the correct concentration and an excellent 501 dispersion of the nanoparticulates throughout the matrix. 502

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 514 as tools for ethylene control but they require expensive materials or techniques and 515 still show a low cost effectiveness. Nano-catalytic degradation of ethylene, and other 516 hazardous materials, is one of the most desirable and challenging goals in the 517 development of environmentally friendly catalysts (Rickerby et al., 2000). It involves 518 the actual destruction of organic contaminants rather than just the transfer from one 519 phase to another. For practical ethylene removal, the best tested catalysts have been 520 Pd and TiO₂ fixed on activated carbon (Rodríguez-Reinoso, 1997). 521

Titanium dioxide (TiO₂) 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 nontoxicity (Hussain et al., 2011). TiO₂ 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).

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540 2.2 Nanoparticles and nanoporous materials for ethylene action inhibition

541

Recent advances in nanotechnology demonstrate the increased attention that is now 542 being paid to the supramolecular assembly of simple components. The design of new 543 biomaterials based on nanoscale structural characteristics can be expected to 544 provide many potential applications. Nano-sized colloidal carriers have recently been 545 developed and proposed for drug delivery, since their use can solubilise poorly water-546 soluble active principles and provide prolonged release, as well as improving their 547 bioavailability and in some cases modifying the kinetic parameters (Cavalli et al., 548 2006). They can also protect active components from degradation. Among colloidal 549 carriers, nanoparticles have in particular been described as a new technological 550 approach (Cavalli et al., 2006). 551

Nanometer-sized silver (Ag⁺) particles (NS) are used in various applications as antimicrobials (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, 559 water purification and various other industrial and non-plant applications (Jain and 560 Pradeep, 2005; Dubas et al., 2006 and Chen and Schluesener, 2008). Their use as a 561 pulse and vase solution treatment for cut flowers is relatively new. Studies have 562 investigated the effectiveness of NS in extending the vase life of some cut flowers, 563 including carnations, gerberas, acacias, and roses (Liu et al., 2009; Solgi et al., 2009; 564 Lü et al., 2010; Liavali and Zarchini, 2012; Liu et al., 2012, Moradi et al., 2012, and 565 Nazemi and Ramezanian, 2013). The positive effect of a NS pulse treatment was 566 attributed to inhibition of bacterial growth in the vase solution and at the cut stem 567 ends. However, physiological activity of Ag⁺ from NS is also a possibility. As with 568 other cations (e.g. K⁺, Ca²⁺), Ag⁺ can have positive effects on plant stem hydraulic 569 conductivity (van leperen, 2007). Also, Ag⁺ is considered to be a general inhibitor of 570 aquaporins (Niemietz and Tyerman, 2002), improving water relations (Lü et al., 571 2010). Besides antibacterial and acidic effects, NS could act as antiethylene agents. 572 Aq+, generally applied as STS, is an effective ethylene action inhibitor (Beyer, 1976; 573 Veen, 1979). Kim et al. (2005) suggested that NS acted as anti-ethylene agents on 574 cut Asiatic hybrid Lilium 'Dream Land' and Oriental hybrid Lilium 'Sibera' (Lü et al., 575 2010). 576

577 Cyclodextrins (CDs) are nanometric biomaterials synthesised by enzymatic action on 578 hydrolysed starch. They have a characteristic toroidal shape, which forms a well-579 defined truncated cone-shaped lipophilic cavity. CDs are able to include compounds 580 whose geometry and polarity are compatible with that of their cavity. Furthermore, 581 chemical modifications of CDs have been studied in an attempt to form inclusion 582 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 583 insoluble multifunctional CD derivatives. These polymers can be obtained by reacting 584 native CDs with a cross-linking agent that, after reaction, exerts its own properties 585 and influences the behaviour of the CD unit. Although insoluble cross-linked CD 586 polymers were first reported a long time ago, the term cyclodextrin nanosponges 587 (CD-NSs) was first used by Li and Ma (1998) to indicate a cross-linked β-CD with 588 organic diisocyanates leading to an insoluble network that showed a very high 589 inclusion constant with several organic pollutants. Generally speaking, CD-NSs are 590 hyper-cross-linked CDs that can be obtained with α , β and γ CDs, either alone or as 591

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).

- 598 Currently, evaluation of the potential for the use of CD-NSs in the field of agriculture 599 appears an important research goal. CD-NSs hold a promising future in various 600 applications such as enhanced product performance, improved thermal, physical, 601 and chemical stability, and extended release and bioavailability.
- In the postharvest context, CD-NSs (patented by Trotta et al., 2006) have been 602 proposed as a delivery system capable of slowing the release of 1-MCP (Devecchi et 603 al., 2009). These have the benefits of requiring reduced active ingredient dosages 604 and reduced number of delivery times, as compared to the gaseous commercial 605 product. In carnation, the inclusion of 1-MCP in a β-CD-NS structure has been shown 606 to be effective not only in prolonging cut flower vase life (5 days more than gaseous 607 1-MCP; Seglie et al., 2011a; Seglie et al., 2011b) but also in controlling Botrytis 608 cinerea damage (a 16% reduction in the development of grey mould; Seglie et al., 609 2012). The superior efficacy in improving postharvest perfomances of 1-MCP 610 included in β-CD-NS has been seen also in a number of other ethylene sensitive 611 species (Anemone coronaria L. multicolor, Ranunculus asiaticus L. 'Minou Abrown', 612 Helianthus annuus L. 'SunrichOrange', Rosa hybrida L. 'Jupiter', Paeonia lactiflora 613 Pall. 'Sarah Bernhardt', and Papaver nudicaule L. multicolor.) (Seglie et al., 2013). 1-614 MCP is a highly unstable and reactive gas that very quickly dimerizes even at room 615 temperature. This dimer has no anti-ethylene activity. Most likely β-CD-NS stabilizes 616 the included 1-MCP thus preserving its properties. 617

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.

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629 **3. Conclusions and Future prospects**

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Although a range of solutions exist currently to reduce the impact of ethylene on 631 postharvest floral longevity through the supply chain, none currently meets all the 632 requirements. However, recent progress in the development of nanotechnological 633 strategies suggests that they have a lot to offer. Nanotechnologies could help to 634 overcome postharvest quality and safety issues by developing user friendly green 635 tools. Nano-scale systems could be applied to cut flowers for ethylene detection in 636 the store environment (nano-metals based sensors) and along the distribution chain 637 (nano-chip labels). They could also be used for ethylene removal (nano-metals for 638 photocatalitic degradation in the warehouse or nanocomposites for scrubbing in 639 active packaging). The use of new natural formulations (e.g. nanosponges) able to 640 increase the bio-availability of the active ingredients has already been shown to 641 enable a reduction in commonly applied concentrations of agrochemicals, helping to 642 minimize the impact of agriculture on the environment and to reduce production 643 costs. However, the efficiency and the economic benefit of applying each strategy to 644 the flower industry needs to be evaluated in the different crop/market contexts. 645

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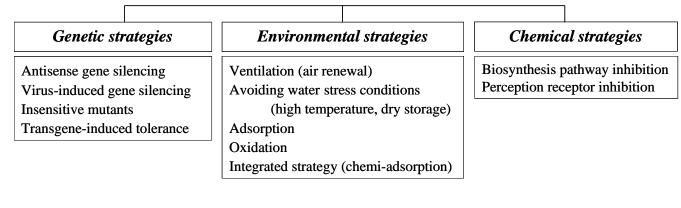
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1078 Figures

Ethylene control strategies

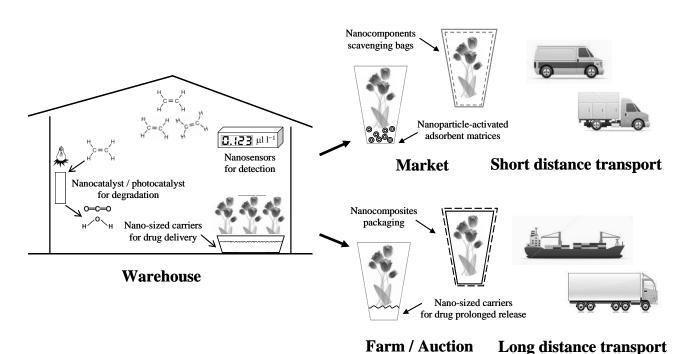


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¹⁰⁸⁰ Figure 1 – Schematic view of ethylene control strategies in production and

1081 distribution chain of ethylene-sensitive plant species.

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