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1 *Types of paper: Review Articles*

2

### 3 **Ethylene control in cut flowers: classical and innovative approaches**

4

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15

#### 16 **Key words**

17 Post harvest, nanosensors, nanocomposites, nanocatalyst, nanoparticles,

18 nanosponges,

19

#### 20 **Highlights**

21 - Potential applications of nanotechnology in ethylene control for cut flowers

22 - Nanoparticle-based sensors for detecting ethylene throughout the distribution chain

23 - Nanocomposites as scavengers for ethylene removal in active packaging

24 - Nanocatalysts to promote ethylene catalytic degradation in the warehouse

25 - Nanoparticles and nanosponges as carriers of drugs for ethylene action inhibition

26

#### 27 **Abstract**

28 Ethylene-mediated premature floral senescence and petal or flower abscission affect

29 postharvest longevity of several species used as cut flowers. Exposure to exogenous

30 or endogenously produced ethylene can be controlled in several ways. These include

31 the use of ethylene biosynthesis inhibitors or ethylene action inhibitors, and ethylene

32 removal technologies. In addition, genetic modification can be very effective in

33 controlling ethylene synthesis and perception. We review here the potential for

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

49

50

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52

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69

#### 70 1. Introduction

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

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

98

99

## 100 1.1 Role of ethylene in floral senescence

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

106

### 107 1.1.1 Ethylene as an endogenous and exogenous regulator

108 Floral lifespan is often terminated by the abscission of petals that are still turgid, or by  
109 petal wilting or withering. In many species, these processes are regulated by the plant  
110 growth regulator, ethylene (van Doorn, 2001; van Doorn and Woltering, 2008) through  
111 changes in endogenous levels. Plant tissues synthesize small amounts of ethylene  
112 ( $0.1-0.2 \mu\text{l Kg}^{-1} \text{h}^{-1}$ ; Martínez-Romero et al., 2007). However ethylene production  
113 changes during plant development and in relation to physiological status (Yang and  
114 Hoffman 1984).

115 In many species exogenous ethylene can also accelerate floral senescence. Ethylene  
116 is produced by many plant tissues (Gane, 1934) and other sources, including bacterial  
117 and fungal fermentation processes, and pyrolysis of hydrocarbons, which releases  
118 ethylene as a component of air pollutants (Cape, 2003), all of which can thus affect the  
119 longevity of cut flowers in the horticultural supply chain. Ethylene is biologically active  
120 at very low concentrations ( $\text{nl-}\mu\text{l l}^{-1}$ ), but there are significant differences in ethylene  
121 sensitivity between species and even cultivars of the same species (Serek et al.,  
122 2006b; Scariot et al., 2008). A detailed classification of flowers based on ethylene  
123 sensitiveness is reported by van Doorn (2001).

124

### 125 1.1.2 Plant species: sensitivity and effects

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

132 wax flower (*Chamelaucium uncinatum*) (Macnish et al., 2000), ethylene induces petal  
133 or flower abscission.

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

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

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

157

## 158 1.2 Ethylene control strategies

159

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

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

172

### 173 *1.2.1 Genetic strategies*

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

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

192 A more effective approach to protecting flowers from exogenous ethylene in the supply  
193 chain is therefore to focus on ethylene perception. Ethylene perception occurs through  
194 a well-conserved signalling pathway and the receptor is encoded by a family of five  
195 genes: *ETR1*, *ETR2*, *EIN4*, *ERS1* and *ERS2* (Yoo et al., 2009). Again an early  
196 discovery was that expression of a mutated *ETR1* gene from *Arabidopsis* (*etr1-1*)  
197 disrupts ethylene signalling in a wide range of heterologous species (Bleecker et al.,

198 1988; Wilkinson et al., 1997), making it an extremely useful tool (Binder, 2008, Serek  
199 et al., 2006a). It has been used successfully in a range of ornamental species to delay  
200 floral senescence including *Petunia* (Clevenger et al., 2004; Clark et al., 1999a;  
201 Gubrium et al., 2000; Wilkinson et al., 1997), *Dianthus* (Bovy et al., 1999), *Campanula*  
202 (Sriskandarajah et al., 2007) and *Kalanchoe* (Sanikhani et al., 2008). Other genes in  
203 the ethylene signalling pathway such as *EIN2*, which is down-stream of the receptor,  
204 have also been down-regulated in ornamental species such as *Petunia* (Shibuya et al.,  
205 2004) resulting in delayed senescence.

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

226 Although these approaches appear to be successful, there has been a lack of  
227 commercialisation in ornamentals and only very few transgenic lines have been  
228 commercialised (Chandler and Sanchez, 2012). One of the barriers is that while there  
229 are hundreds of ornamental cut flower species and thousands of varieties, only about  
230 fifty ornamental species are transformable (Chandler and Sanchez, 2012). A further



231 barrier is ascribed to the cost and complexity of the regulatory process and lack of  
232 harmonisation of the regulations across different world markets. Furthermore, despite  
233 being the largest market for ornamentals, the European regulatory environment is one  
234 of the most stringent. Alternative strategies are also therefore still required.

235

### 236 *1.2.2 Environmental strategies*

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

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

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

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

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

285 Another strategy is oxidation. Inert matrices (e.g. alumina or silica gel) impregnated  
286 with potassium permanganate ( $\text{KMnO}_4$ ), can be used as oxidising agents (Terry et al.,  
287 2007). However, performance of  $\text{KMnO}_4$  depends on the percentage of active agent  
288 per matrix weight (usually 4 to 6%) and surface area of the substrate (Poças et al.,  
289 2008). In addition, in common with most of the ethylene scavengers,  $\text{KMnO}_4$  has  
290 limited long-term efficacy in environments with high relative humidity (RH) (e.g. cold  
291 chambers, packaging, etc.) (Terry et al., 2007). Ozone ( $\text{O}_3$ ) is an alternative gaseous  
292 oxidant, with good solubility in water and reactivity. Ozone acts as a powerful, residue-  
293 free ethylene oxidant and microbial disinfectant, which does not impair product  
294 appearance, texture, or scent. However it is highly unstable and decomposes easily  
295 into  $\text{O}_2$  (Dickson et al., 1992). Furthermore, even though it has been listed as a GRAS

296 (generally recognized as safe) material by the US Food and Drug Administration  
297 (FDA), its application is strictly regulated (Mahapatra et al., 2005).

298 The combination of an adsorbent with an oxidizer or catalyst (chemi-adsorption)  
299 enhances the efficacy of the two single strategies. Indeed, the use of some catalysts  
300 (palladium Pd, titanium Ti, copper Cu, rhodium Rh and cobalt Co) have also been  
301 shown to be effective in ethylene removal, by oxidising it to CO<sub>2</sub> and H<sub>2</sub>O, even at low  
302 temperature and high RH (Conte et al., 1992; Maneerat et al., 2003). For example,  
303 results obtained by combining activated carbon with Pd have been far superior to  
304 KMnO<sub>4</sub>-based scavengers at room temperature (20 °C) (Bailén et al., 2007; Terry et  
305 al., 2007). Pd fixed on activated carbon increased the efficiency of ethylene adsorbtion  
306 compared to activated carbon alone, even at low concentration (1% in weight), making  
307 this strategy sustainable for practical applications in common packaging and modified  
308 atmosphere packaging (MAP), despite the high cost of Pd (Martínez-Romero et al.,  
309 2007). However, this kind of system has several disadvantages, including the large  
310 quantity of adsorbent + catalyst required (due to adsorption of other environmental  
311 gases and the subsequent loss of efficacy over time), the requirement to reposition the  
312 material, and non-continuous operation (since regeneration of the adsorbent is  
313 necessary) (Martínez-Romero et al., 2009).

314 A refinement to the adsorbent + catalyst strategy that can be used to remove ethylene  
315 continuously has been developed based on activated carbon-1% Pd and the  
316 application of short heat pulses (Martínez-Romero et al., 2009). This system allows an  
317 increase in the rate of ethylene adsorbtion and oxidation (96-99% at 150-200 °C) and  
318 the elimination of deposits of other gases on the activated carbon, avoiding system  
319 saturation (auto-regeneration). It thus compares favourably to other non heated  
320 adsorbent-catalyst systems, with low CO<sub>2</sub> accumulation and without affecting the  
321 temperature of the storage environment. Silver (Ag) ions also appear attractive as a  
322 catalyst, because of their photoactivity, photocatalysis, and antibacterial activity  
323 (Verykios et al., 1980).

324 In summary, ventilation and air temperature control are commonly used during  
325 postharvest storage and transport of most cut flowers, together with adsorbent or  
326 oxidizers, while “ozonators” and catalytic degradation reactors are less widely used.  
327 However, recent advances in technology promise to expand the use of catalytic

328 degradation in ethylene control in the floriculture industry (e.g. the carbon-heat hybrid  
329 ethylene scrubber; Martínez-Romero et al., 2009).

330

### 331 *1.2.3 Chemical strategies*

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

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

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

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

360 commercial use (Sisler et al., 1990). Similarly, instability and explosive characteristics  
361 of DACP make it an unlikely candidate for commercial use (Serek et al., 2006b).  
362 1-MCP was the first patented non-toxic ethylene action inhibitor (Sisler and  
363 Blankenship, 1996). 1-MCP treatment conditions and effects on floricultural crops have  
364 been reviewed by Blankenship and Dole (2003). Its high efficacy has been well  
365 documented in a range of ornamental species and it is now widely used commercially  
366 under the trade name of EthylBloc® and SmartFresh™ (Serek et al., 2006b). However,  
367 the gaseous nature of 1-MCP leads to difficulties with its use due to three key factors:  
368 (i) plant material must be kept in enclosed areas to prevent gas leakage, (ii) the effect  
369 of 1-MCP can be transitory in some plants, depending on the species, the  
370 concentrations, and lighting (Sisler et al., 1996a, b; Blankenship and Dole 2003;  
371 Kebenei et al., 2003; Feng et al., 2004; Apelbaum et al., 2008), thus some ornamentals  
372 require continuous or repeated applications, (Serek and Sisler, 2005; Serek et al.,  
373 2006b) and (iii) and the action of commercial formulations of 1-MCP appears to be  
374 strongly reduced by treatment temperature (0–5°C) and by the presence of exogenous  
375 ethylene (Seglie et al., 2011a; Çelikel and Reid, 2002; Reid and Çelikel, 2008).  
376 Furthermore, many conventional 1-MCP delivery vehicles, such as cyclopropenes and  
377 cyclodextrins, have low preservative efficiency and, consequently, require high  
378 concentrations of active ingredients to be effective. These levels may induce side  
379 effects due to the high input levels (Sisler et al., 1996a, b, 1999). Advances have  
380 occurred to counter some of these limitations by developing 1-MCP-based compounds  
381 that can be applied in non-volatile formulations. Different cyclopropene salt compounds  
382 such as N,N-dipropyl(1-cyclopropenylmethyl)amine (DPCA) have been recently  
383 synthesized (Sisler et al., 2009) and used to protect several ornamentals against  
384 ethylene (Seglie et al., 2010). Cyclopropene salt compounds differ amongst each other  
385 in their chemical structure, but they all have a methyl group in the 1-position, onto  
386 which an amine is substituted. Such compounds can be used as a gas in a confined  
387 space or as a salt in open spaces. Moreover, recently, the company Floralife  
388 (Walterboro, SC) has released a novel treatment system 1-MCP sachets resembling  
389 tea bags. The bags are dipped in water just before being placed within a packed box;  
390 the water diffuses through the bag, and the 1-MCP in it is released into the air within  
391 the box. Preliminary experiments have shown this technique to be quite effective (Reid  
392 and Çelikel, 2008).

393

## 394 **2. Nanotechnology for ethylene control**

395

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

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

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

417

### 418 **2.1 Nanotechnology for ethylene detection and removal in the postharvest environment**

419

#### 420 *2.1.1 Sensors using nanoparticles for the detection of ethylene*

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

424 The most common nano-material used for detection in ethylene sensors is tin dioxide  
425 (stannic oxide, SnO<sub>2</sub>) (Ivanov et al., 2005; Agarwal et al., 2012), others are tungsten

426 trioxide ( $\text{WO}_3$ , Pitcher et al., 2003), palladium (Pd, Pietrucha and Lalevic, 1988),  
427 platinum (Pt, Winquist and Lundström, 1987), titanium dioxide ( $\text{TiO}_2$ , Zhang et al.,  
428 2002), and zinc oxide ( $\text{ZnO}$ , Kang et al., 2004).

429 In more sophisticated versions,  $\text{WO}_3$ - $\text{SnO}_2$  binary oxide, with uniform distribution of  
430 nano- $\text{WO}_3$  within a  $\text{SnO}_2$  particle-based material, has been developed successfully  
431 (Pimtong-Ngam et al., 2007). Similarly, nano-Au/ $\text{Co}_3\text{O}_4$ , with gold catalyst  
432 nanoparticles dispersed on a nano- $\text{Co}_3\text{O}_4$  support surface, showed great potential,  
433 particularly for indoor environmental control of ethylene traces (Li et al., 2008). Most of  
434 these materials are used in resistor-based devices, where their conductivity increases  
435 or decreases as an effect of the exposure to different ethylene concentrations.

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

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

454 Gas sensors containing nanostructures such as nanowires, e.g. the electronic  
455 detectors called electronic nose or e-nose, identify the odorant mimicking natural  
456 olfaction and estimate its concentration (Gardner and Bartlett, 1999). Sensors based  
457 on e-nose technology allow detection of the presence of ethylene in food products,  
458 because of contamination or spoilage (Valdés et al., 2009). Information from e-noses

459 on fruit physiological states, based on changes in released volatiles, can be applied to  
460 retard the ripening process through exposure of the fruit to inhibitors (such as  
461 cyclopropene compounds as ethylene-receptor blockers) at the appropriate time,  
462 adjustments in storage conditions to preclude ethylene accumulation, and removal of  
463 bruised or damaged fruits, over-producing ethylene (Wilson and Baietto, 2009).  
464 Nanomaterial-based sensors are widely applied in post harvest management of fruits  
465 (e.g. climacteric fruits like apples and peaches) and in the food industry (e.g. packaging  
466 of vegetables) (Cristescu et al., 2012). Nanosensors could therefore also help to  
467 prolong vase life of cut flowers, by enabling monitoring of ethylene concentrations in  
468 storage rooms of large growers and wholesale markets. However, a cost-benefit  
469 analysis is necessary to evaluate if this extra cost would be compensated by the  
470 extension of cut flower vase life in the different flower species and the specific market  
471 context. In addition, it has to be taken into account that monitoring ethylene levels in  
472 the supply chain would be useful only if the integrated ethylene exposure can be  
473 calculated and suitable data on the specific sensitivity of flowers to different levels of  
474 ethylene are available. In this respect, further research is needed in order to clarify the  
475 mechanisms of response to ethylene in the different plant species (reaction to a  
476 threshold value or an integral amount of ethylene). Furthermore, differences in  
477 sensitivity between species and even between varieties means that a very  
478 sophisticated system would be required which may not ultimately be cost-effective and  
479 may have limited applicability with mixed batches.

480

### 481 *2.1.2 Nanocomposites and nanocatalyst for ethylene removal and photodegradation*

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



492 2009) to include in classical packaging. Nanoparticulates work as small physical  
493 barriers to the movement of gas molecules, by obstructing the path of the gas through  
494 the material. Furthermore, they have a relatively larger surface area than larger fillers,  
495 which favours filler-matrix interactions and the performance of the composite, acting  
496 as nano-reinforcements. However, achieving optimal barrier and mechanical  
497 performance requires the correct concentration and an excellent dispersion of the  
498 nanoparticulates throughout the matrix.

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

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

510 Some alternatives to active packaging (e.g. catalytic degradation) look very attractive  
511 as tools for ethylene control but they require expensive materials or techniques and  
512 still show a low cost effectiveness. Nano-catalytic degradation of ethylene, and other  
513 hazardous materials, is one of the most desirable and challenging goals in the  
514 development of environmentally friendly catalysts (Rickerby et al., 2000). It involves  
515 the actual destruction of organic contaminants rather than just the transfer from one  
516 phase to another. For practical ethylene removal, the best tested catalysts have been  
517 Pd and TiO<sub>2</sub> fixed on activated carbon (Rodríguez-Reinoso, 1997).

518 Titanium dioxide (TiO<sub>2</sub>) has been the focus for light-activated photocatalytic  
519 degradation under ultraviolet (UV) irradiation, either from natural (sun) or artificial  
520 (lamps), because of its physical and chemical stability, low cost, availability and non-  
521 toxicity (Hussain et al., 2011). TiO<sub>2</sub> action is unaffected by relative humidity and is  
522 efficient at room temperature, however the constant need for UV light represents a  
523 limiting factor.

524 Silver (Ag) ions also show photoactivity, semiconductor photocatalysis, and  
525 antibacterial activity: nano-Ag absorbs and decomposes ethylene and can have more  
526 effective antibacterial activity than Ag (Hu and Fu, 2003). Thus, packaging films  
527 incorporating nano- Ag or TiO<sub>2</sub> (e.g. nanocomposite polyethylene film) contribute to  
528 preserve quality of fruits and vegetables, retarding senescence and decreasing  
529 microbial growth.

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

535

## 536 2.2 Nanoparticles and nanoporous materials for ethylene action inhibition

537

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

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

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

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

578 One approach is to synthesize cross-linked CD-based polymers in order to prepare  
579 insoluble multifunctional CD derivatives. These polymers can be obtained by reacting  
580 native CDs with a cross-linking agent that, after reaction, exerts its own properties and  
581 influences the behaviour of the CD unit. Although insoluble cross-linked CD polymers  
582 were first reported a long time ago, the term cyclodextrin nanosponges (CD-NSs) was  
583 first used by Li and Ma (1998) to indicate a cross-linked β-CD with organic  
584 diisocyanates leading to an insoluble network that showed a very high inclusion  
585 constant with several organic pollutants. Generally speaking, CD-NSs are hyper-cross-  
586 linked CDs that can be obtained with α, β and γ CDs, either alone or as mixtures  
587 containing relevant amounts of linear dextrin, cross-linked with a suitable cross-linking  
588 agent. CD-NSs were initially used for removing persistent organic pollutants (POPs) in  
589 water purification (Li and Ma, 1999; Arkas et al., 2006). Then, further studies were

590 carried out in the preparation of cosmetics. Lately, medical and pharmaceutical  
591 applications have been of particular relevance, in which CD-NSs are used as carries  
592 for drug delivery (Trotta et al., 2012; Trotta, 2011).

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

597 In the postharvest context, CD-NSs (patented by Trotta et al., 2006) have been  
598 proposed as a delivery system capable of slowing the release of 1-MCP (Devecchi et  
599 al., 2009). These have the benefits of requiring reduced active ingredient dosages and  
600 reduced number of delivery times, as compared to the gaseous commercial product.

601 In carnation, the inclusion of 1-MCP in a  $\beta$ -CD-NS structure has been shown to be  
602 effective not only in prolonging cut flower vase life (5 days more than gaseous 1-MCP;  
603 Seglie et al., 2011a; Seglie et al., 2011b) but also in controlling *Botrytis cinerea* damage  
604 (a 16% reduction in the development of grey mould; Seglie et al., 2012). The superior  
605 efficacy in improving postharvest performances of 1-MCP included in  $\beta$ -CD-NS has  
606 been seen also in a number of other ethylene sensitive species (*Anemone coronaria*  
607 L. multicolor, *Ranunculus asiaticus* L. 'Minou Abrown', *Helianthus annuus* L.  
608 'SunrichOrange', *Rosa hybrida* L. 'Jupiter', *Paeonia lactiflora* Pall. 'Sarah Bernhardt',  
609 and *Papaver nudicaule* L. multicolor.) (Seglie et al., 2013). 1-MCP is a highly unstable  
610 and reactive gas that very quickly dimerizes even at room temperature. This dimer has  
611 no anti-ethylene activity. Most likely  $\beta$ -CD-NS stabilizes the included 1-MCP thus  
612 preserving its properties.

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

623

### 624 **3. Conclusions and Future prospects**

625

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

641

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647

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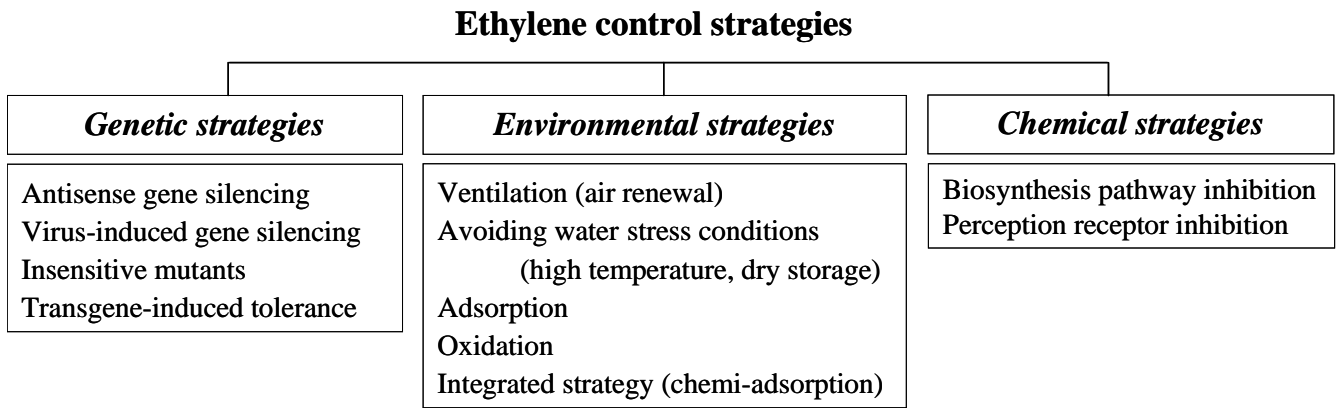
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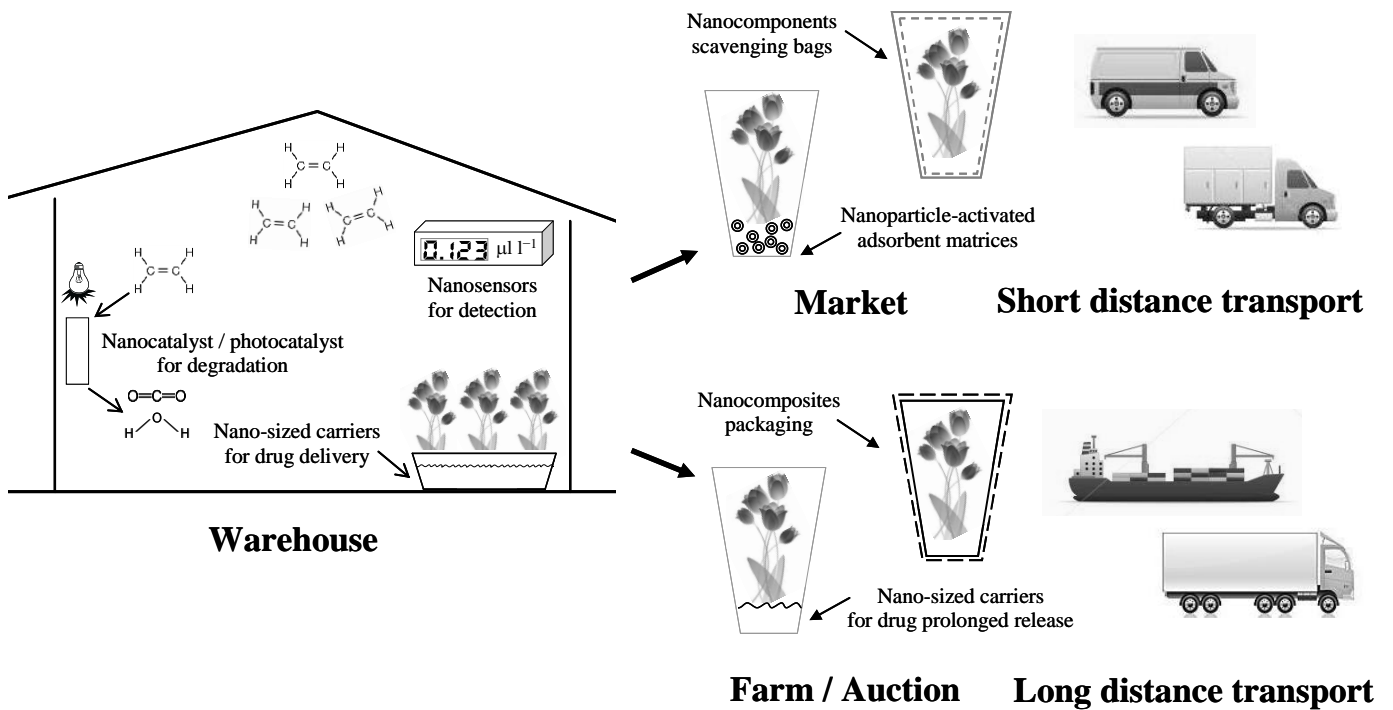
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1068 **Figure 1** – Schematic view of ethylene control strategies in production and  
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 1073 **Figure 2** – Example of futuristic nanotechnology-based system for ethylene control in  
 1074 ethylene-sensitive cut flowers.  
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