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

2

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13

## 14 **Key words**

15 Post harvest, nanosensors, nanocomposites, nanocatalyst, nanoparticles,

16 nanosponges,

17

## 18 **Highlights**

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

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

21 - Nanocomposites as scavengers for ethylene removal in active packaging

22 - Nanocatalysts to promote ethylene catalytic degradation in the warehouse

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

24

## 25 **Abstract**

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

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

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

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

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

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

32 applications of nanotechnology to control ethylene levels and postharvest

33 management in the flower industry. Already nanosponges have been shown to

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

47

48

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50

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67

## 68 **1. Introduction**

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

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

96

97

### 98 **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).

104

#### 105 *1.1.1 Ethylene as an endogenous and exogenous regulator*

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

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

122

#### 123 *1.1.2 Plant species: sensitivity and effects*

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

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

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

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

156

## 157 1.2 Ethylene control strategies

158

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

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

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

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



197 1988; Wilkinson et al., 1997), making it an extremely useful tool (Binder, 2008, Serek  
198 et al., 2006a). It has been used successfully in a range of ornamental species to  
199 delay floral senescence including *Petunia* (Clevenger et al., 2004; Clark et al., 1999a;  
200 Gubrium et al., 2000; Wilkinson et al., 1997), *Dianthus* (Bovy et al., 1999),  
201 *Campanula* (Sriskandarajah et al., 2007) and *Kalanchoe* (Sanikhani et al., 2008).  
202 Other genes in the ethylene signalling pathway such as *EIN2*, which is down-stream  
203 of the receptor, have also been down-regulated in ornamental species such as  
204 *Petunia* (Shibuya et al., 2004) resulting in delayed senescence.

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

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

235

### 236 *1.2.2 Environmental strategies*

237 In many situations, considerable ethylene emission occurs throughout the  
238 horticultural distribution chain, such as in producer or market refrigerators and  
239 storage chambers, inside packaging, and during transportation (Martínez-Romero et  
240 al., 2007). This ethylene comes from normal emission from plant organs or external  
241 sources, such as micro organism metabolism and pyrolysis of hydrocarbons in  
242 internal combustion 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  
248 ripening processes of climacteric fruits (Ivanov et al., 2005). In fact air concentrations  
249 higher 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  
255 or 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  
261 99% RH are the recommended conditions for these cut flowers and forced air cooling  
262 is the common method for pre-cooling products prior to storage (Reid and Jiang,

263 2012).

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

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

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

296 product appearance, texture, or scent. However it is highly unstable and decomposes  
297 easily into O<sub>2</sub> (Dickson et al., 1992). Furthermore, even though it has been listed as a  
298 GRAS (generally recognized as safe) material by the US Food and Drug  
299 Administration (FDA), its application is strictly regulated (Mahapatra et al., 2005).

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

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

326 In summary, ventilation and air temperature control are commonly used during  
327 postharvest storage and transport of most cut flowers, together with adsorbers or  
328 oxidizers, while “ozonators” and catalytic degradation reactors are less widely used.

329 However, recent advances in technology promise to expand the use of catalytic  
330 degradation in ethylene control in the floriculture industry (e.g. the carbon-heat hybrid  
331 ethylene scrubber; Martínez-Romero et al., 2009).

332

### 333 *1.2.3 Chemical strategies*

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

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

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

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

362 commercial use (Sisler et al., 1990). Similarly, instability and explosive characteristics  
363 of DACP make it an unlikely candidate for commercial use (Serek et al., 2006b).

364 1-MCP was the first patented non-toxic ethylene action inhibitor (Sisler and  
365 Blankenship, 1996). 1-MCP treatment conditions and effects on floricultural crops  
366 have been reviewed by Blankenship and Dole (2003). Its high efficacy has been well  
367 documented in a range of ornamental species and it is now widely used commercially  
368 under the trade name of EthylBloc® and SmartFresh™ (Serek et al., 2006b).

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

395

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

397

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

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

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

419

420 **2.1 Nanotechnology for ethylene detection and removal in the postharvest**  
421 **environment**

422

### 423 *2.1.1 Sensors using nanoparticles for the detection of ethylene*

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

427 The most common nano-material used for detection in ethylene sensors is tin dioxide  
428 (stannic oxide, SnO<sub>2</sub>) (Ivanov et al., 2005; Agarwal et al., 2012), others are tungsten  
429 trioxide (WO<sub>3</sub>, Pitcher et al., 2003), palladium (Pd, Pietrucha and Lalevic, 1988),  
430 platinum (Pt, Winqvist and Lundström, 1987), titanium dioxide (TiO<sub>2</sub>, Zhang et al.,  
431 2002), and zinc oxide (ZnO, Kang et al., 2004).

432 In more sophisticated versions, WO<sub>3</sub>-SnO<sub>2</sub> binary oxide, with uniform distribution of  
433 nano-WO<sub>3</sub> within a SnO<sub>2</sub> particle-based material, has been developed successfully  
434 (Pimtong-Ngam et al., 2007). Similarly, nano-Au/Co<sub>3</sub>O<sub>4</sub>, with gold catalyst  
435 nanoparticles dispersed on a nano-Co<sub>3</sub>O<sub>4</sub> support surface, showed great potential,  
436 particularly for indoor environmental control of ethylene traces (Li et al., 2008). Most  
437 of these materials are used in resistor-based devices, where their conductivity  
438 increases or decreases as an effect of the exposure to different ethylene  
439 concentrations.

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

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

458 Gas sensors containing nanostructures such as nanowires, e.g. the electronic  
459 detectors called electronic nose or e-nose, identify the odorant mimicking natural



460 olfaction and estimate its concentration (Gardner and Bartlett, 1999). Sensors based  
461 on e-nose technology allow detection of the presence of ethylene in food products,  
462 because of contamination or spoilage (Valdés et al., 2009). Information from e-noses  
463 on fruit physiological states, based on changes in released volatiles, can be applied  
464 to retard the ripening process through exposure of the fruit to inhibitors (such as  
465 cyclopropene compounds as ethylene-receptor blockers) at the appropriate time,  
466 adjustments in storage conditions to preclude ethylene accumulation, and removal of  
467 bruised or damaged fruits, over-producing ethylene (Wilson and Baietto, 2009).

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

484

#### 485 *2.1.2 Nanocomposites and nanocatalyst for ethylene removal and photodegradation*

486 Loss of quality and freshness of plant products during the time required for  
487 commercialization and consumption can be contained by means of the right selection  
488 of materials and packaging technologies, able to maintain the desired atmosphere. In  
489 this respect, nanotechnology can provide effective scavengers with selective ability to  
490 remove different gases (e.g. oxygen, ethylene). In particular, inclusion of nano-scale  
491 fillers (e.g. Pd) within the matrix can make plastic films more impermeable to  
492 ethylene (Neethirajan and Jayas, 2011). These nano-components help to create

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

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

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

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

522 Titanium dioxide (TiO<sub>2</sub>) has been the focus for light-activated photocatalytic  
523 degradation under ultraviolet (UV) irradiation, either from natural (sun) or artificial  
524 (lamps), because of its physical and chemical stability, low cost, availability and non-  
525 toxicity (Hussain et al., 2011). TiO<sub>2</sub> action is unaffected by relative humidity and is

526 efficient at room temperature, however the constant need for UV light represents a  
527 limiting factor.

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

534 Application of nanocomposites and nanocatalysts in floriculture is still limited,  
535 however current advances in packaging materials and formats (reviewed by Rhim et  
536 al., 2013) and successful tests on photocatalytic reactor prototypes (Hussain et al.,  
537 2011; Li et al., 2008) demonstrate how these technologies are potentially  
538 economically viable for commercial application to cut flowers (Figure 2).

539

## 540 2.2 Nanoparticles and nanoporous materials for ethylene action inhibition

541

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

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

559 et al., 2005). Use of NS is becoming increasingly widespread in medicine, fabrics,  
560 water purification and various other industrial and non-plant applications (Jain and  
561 Pradeep, 2005; Dubas et al., 2006 and Chen and Schluesener, 2008). Their use as a  
562 pulse and vase solution treatment for cut flowers is relatively new. Studies have  
563 investigated the effectiveness of NS in extending the vase life of some cut flowers,  
564 including carnations, gerberas, acacias, and roses (Liu et al., 2009; Solgi et al., 2009;  
565 Lü et al., 2010; Liavali and Zarchini, 2012; Liu et al., 2012, Moradi et al., 2012, and  
566 Nazemi and Ramezani, 2013). The positive effect of a NS pulse treatment was  
567 attributed to inhibition of bacterial growth in the vase solution and at the cut stem  
568 ends. However, physiological activity of  $\text{Ag}^+$  from NS is also a possibility. As with  
569 other cations (e.g.  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ),  $\text{Ag}^+$  can have positive effects on plant stem hydraulic  
570 conductivity (van Ieperen, 2007). Also,  $\text{Ag}^+$  is considered to be a general inhibitor of  
571 aquaporins (Niemietz and Tyerman, 2002), improving water relations (Lü et al.,  
572 2010). Besides antibacterial and acidic effects, NS could act as antiethylene agents.  
573  $\text{Ag}^+$ , generally applied as STS, is an effective ethylene action inhibitor (Beyer, 1976;  
574 Veen, 1979). Kim et al. (2005) suggested that NS acted as anti-ethylene agents on  
575 cut Asiatic hybrid *Lilium* 'Dream Land' and Oriental hybrid *Lilium* 'Sibera' (Lü et al.,  
576 2010).

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).

583 One approach is to synthesize cross-linked CD-based polymers in order to prepare  
584 insoluble multifunctional CD derivatives. These polymers can be obtained by reacting  
585 native CDs with a cross-linking agent that, after reaction, exerts its own properties  
586 and influences the behaviour of the CD unit. Although insoluble cross-linked CD  
587 polymers were first reported a long time ago, the term cyclodextrin nanosponges  
588 (CD-NSs) was first used by Li and Ma (1998) to indicate a cross-linked  $\beta$ -CD with  
589 organic diisocyanates leading to an insoluble network that showed a very high  
590 inclusion constant with several organic pollutants. Generally speaking, CD-NSs are  
591 hyper-cross-linked CDs that can be obtained with  $\alpha$ ,  $\beta$  and  $\gamma$  CDs, either alone or as

592 mixtures containing relevant amounts of linear dextrin, cross-linked with a suitable  
593 cross-linking agent. CD-NSs were initially used for removing persistent organic  
594 pollutants (POPs) in water purification (Li and Ma, 1999; Arkas et al., 2006). Then,  
595 further studies were carried out in the preparation of cosmetics. Lately, medical and  
596 pharmaceutical applications have been of particular relevance, in which CD-NSs are  
597 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.

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

618 Therefore, 1-MCP included in  $\beta$ -CD-NS may be a promising user-friendly formulation,  
619 with low environmental impact, for prolonging the shelf life and controlling fungal  
620 diseases of cut flowers in the postharvest environment, although the mechanism of  
621 action needs further elucidation (Seglie et al., 2013). This new formulation appears  
622 moreover to have important economic implications: its application does not require  
623 an air-tight environment, allowing easier and faster open-space application, a major  
624 advantage for field production in ornamental nurseries/gardens. However, future

625 commercial use of 1-MCP included in  $\beta$ -CD-NS will require more development to  
626 optimize chemical concentration and to evaluate this compound on an extended  
627 number of plant species in a range of environments.

628

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

630

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

646

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652

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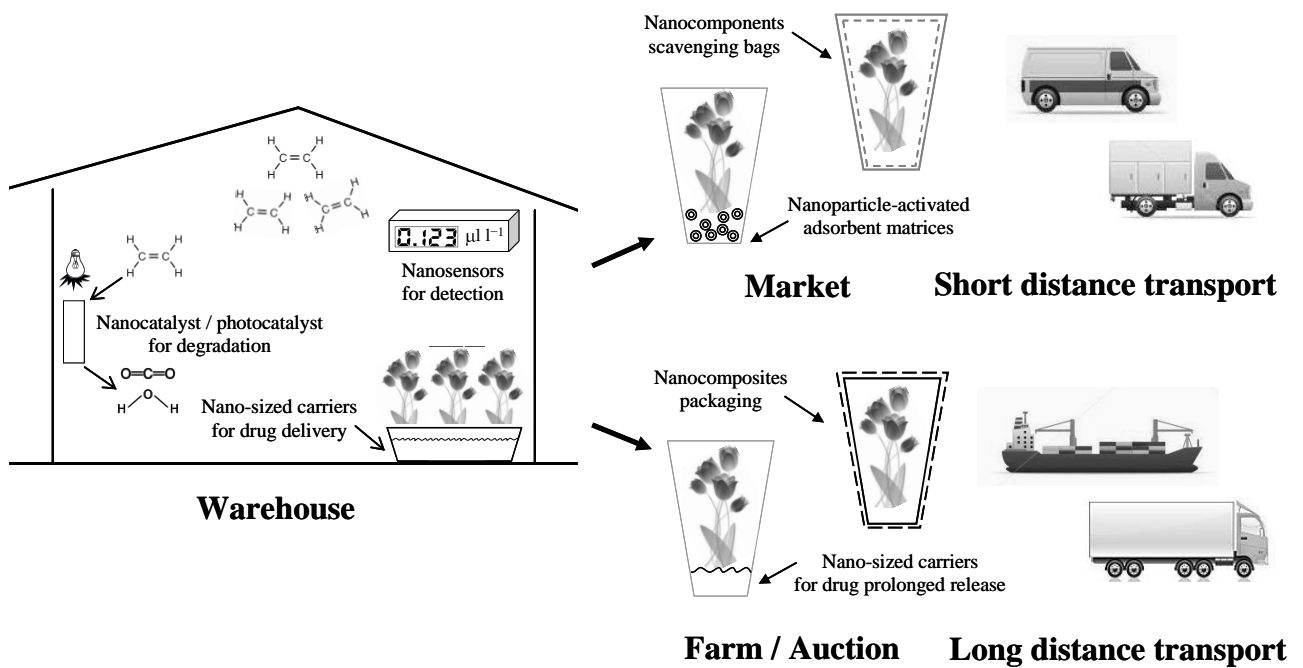
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**Ethylene control strategies**

<i>Genetic strategies</i>	<i>Environmental strategies</i>	<i>Chemical strategies</i>
Antisense gene silencing Virus-induced gene silencing Insensitive mutants Transgene-induced tolerance	Ventilation (air renewal) Avoiding water stress conditions (high temperature, dry storage) Adsorption Oxidation Integrated strategy (chemi-adsorption)	Biosynthesis pathway inhibition Perception receptor inhibition

1079

1080 Figure 1 – Schematic view of ethylene control strategies in production and  
 1081 distribution chain of ethylene-sensitive plant species.  
 1082



1083

1084 Figure 2 – Example of futuristic nanotechnology-based system for ethylene control in  
 1085 ethylene-sensitive cut flowers.