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Citation for final published version:

Scariot, Valentina, Paradiso, Roberta, Rogers, Hilary Joan ORCID: <https://orcid.org/0000-0003-3830-5857> and De Pascale, Stefania 2014. Ethylene control in cut flowers: classical and innovative approaches. *Postharvest Biology and Technology* 97 , pp. 83-92. 10.1016/j.postharvbio.2014.06.010 file

Publishers page: <http://dx.doi.org/10.1016/j.postharvbio.2014.06.01...>
<<http://dx.doi.org/10.1016/j.postharvbio.2014.06.010>>

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1 *Types of paper: Review Articles*

2

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

4

5 Valentina Scariot¹, Roberta Paradiso², Hilary Rogers³, Stefania De Pascale²

6 ¹ Department of Agricultural, Forest and Food Sciences University of Turin (Italy)

7 ² Department of Agricultural Sciences, University of Naples (Italy)

8 ³ School of Biosciences Cardiff University (UK)

9

10 Corresponding author

11 Roberta Paradiso, roberta.paradiso@unina.it

12 Department of Agricultural Sciences, University of Naples Federico II, Via Università,

13 100

14 80055 - Portici - Naples (Italy). Phone N. +39 081 2539135

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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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 (KMnO_4), can be used as oxidising agents (Terry et al.,
287 2007). However, performance of 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, 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 (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 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 CO₂ and H₂O, 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 KMnO₄-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 CO₂ 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, SnO₂) (Ivanov et al., 2005; Agarwal et al., 2012), others are tungsten

426 trioxide (WO_3 , Pitcher et al., 2003), palladium (Pd, Pietrucha and Lalevic, 1988),
427 platinum (Pt, Winquist and Lundström, 1987), titanium dioxide (TiO_2 , Zhang et al.,
428 2002), and zinc oxide (ZnO , Kang et al., 2004).

429 In more sophisticated versions, WO_3 - SnO_2 binary oxide, with uniform distribution of
430 nano- WO_3 within a SnO_2 particle-based material, has been developed successfully
431 (Pimtong-Ngam et al., 2007). Similarly, nano-Au/ Co_3O_4 , with gold catalyst
432 nanoparticles dispersed on a nano- Co_3O_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 TiO₂ fixed on activated carbon (Rodríguez-Reinoso, 1997).

518 Titanium dioxide (TiO₂) 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). TiO₂ 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 TiO₂ (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⁺) 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⁺ (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⁺ from NS is also a possibility. As with other cations
565 (e.g. K⁺, Ca²⁺), Ag⁺ can have positive effects on plant stem hydraulic conductivity (van
566 leperen, 2007). Also, Ag⁺ 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⁺, 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 β -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 β -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 β -CD-NS stabilizes the included 1-MCP thus
612 preserving its properties.

613 Therefore, 1-MCP included in β -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 β -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

642 **Acknowledgment**

643 This research was supported by the research project PRIN 2007 "Post-production
644 physiology of ornamentals and quality maintenance technologies" founded by the
645 Italian Ministry of University and Research. The authors contributed to this study in
646 equal measure.

647

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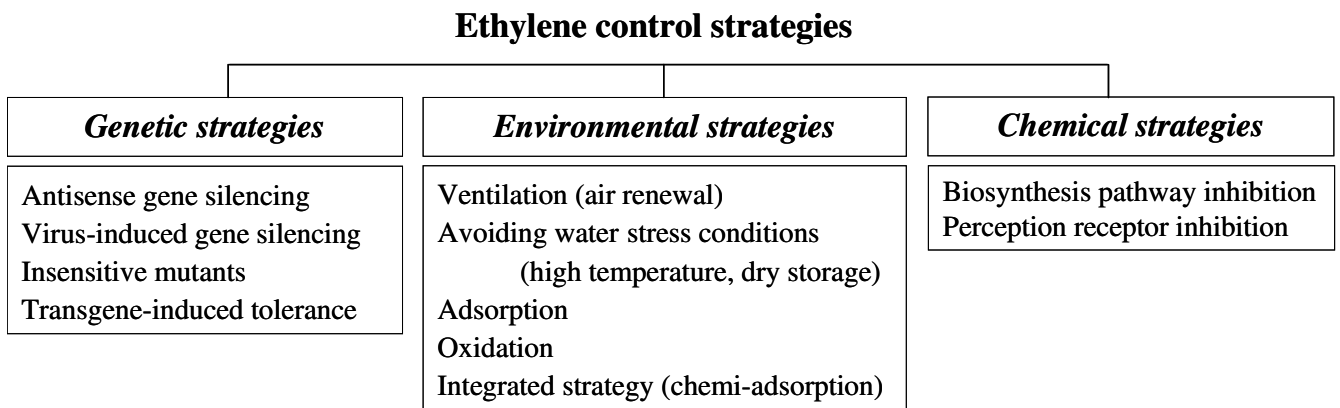
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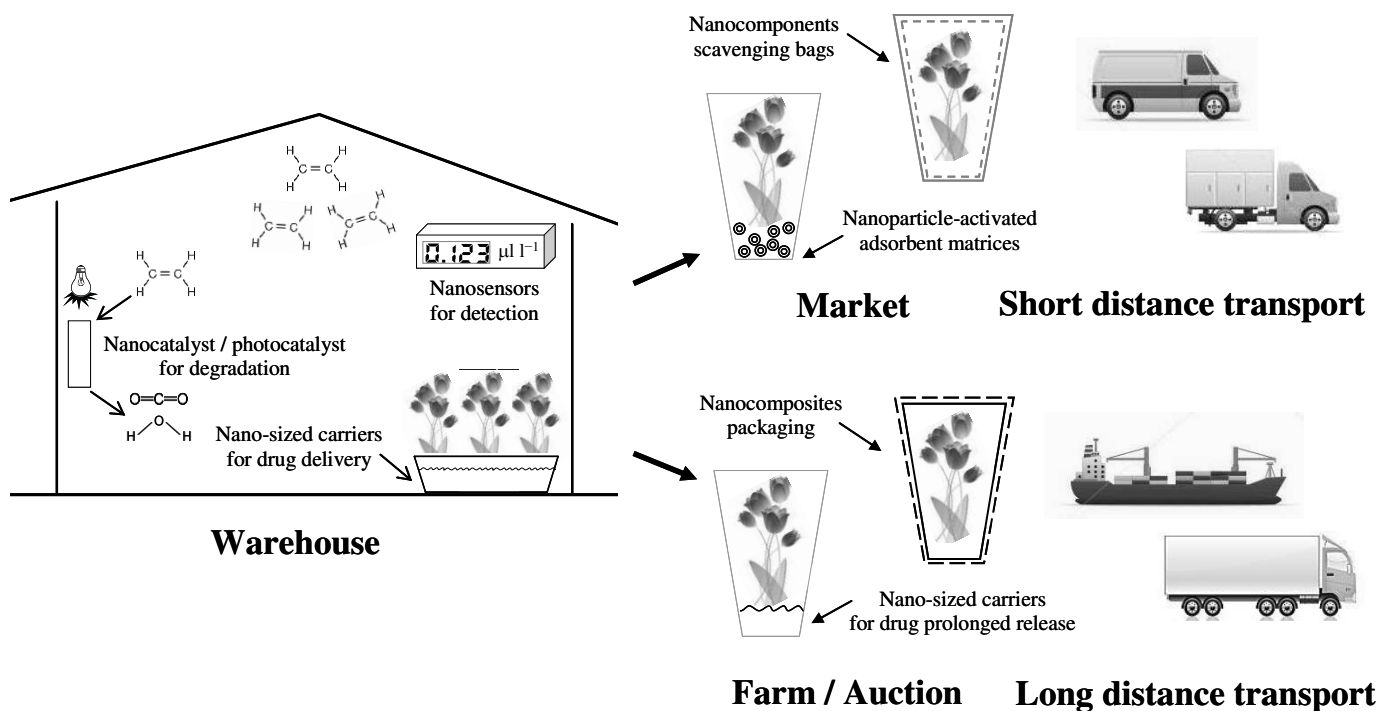
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1068 **Figure 1** – Schematic view of ethylene control strategies in production and
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 1073 **Figure 2** – Example of futuristic nanotechnology-based system for ethylene control in
 1074 ethylene-sensitive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