Another Look at Interpreting Research to Manage the Effects of Ethylene in Ambient Air

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ABSTRACT
Ethylene is a plant hormone that regulates many aspects of plant growth and development. The gas also occurs in the atmosphere from both natural and artificial sources. Sufficient concentrations of ethylene in air can cause damage by adverse effects on flower senescence, abscission, fruit ripening, and other processes. Recent discoveries regarding ethylene receptors and signal transduction now provide a firm basis to understand the dynamics of ethylene effects. Receptor proteins act as negative regulators of ethylene signaling so that the concentration of both ethylene and receptor proteins influence the plant response. Studies with carefully controlled levels of ethylene and environmental conditions have shown that plants grown outdoors are less sensitive to ethylene than those grown and exposed under artificial conditions. Additionally, intermittent exposures, common in many circumstances, allow time for ethylene to diffuse away from receptors. During flower senescence and fruit ripening, synthesis of ethylene often accelerates, reducing the effectiveness of environmental ethylene on plant behavior. Workers interested in promoting crop yields and extending the life of flowers, plants, and vegetative produce can be most effective if they evaluate species characteristics, the nature of the environment, and the sources of ethylene for their location. A universal air quality standard for ethylene is not advisable.

Ethylene is a plant hormone (Abeles, 1973; Abeles et al., 1992). It is the only recognized plant hormone that is a gas. Ethylene modifies many processes throughout the life span of plants and thus is of broad interest in botany, agronomy, and horticulture. Ethylene also occurs as a contaminant in air. Sources include both natural and anthropomorphic processes such as combustion of organic materials (volcanoes and forest fires), vehicle exhausts, and various industrial processes and as a byproduct of plant, animal, and microbe metabolism. When ethylene in air reaches or exceeds physiologically relevant levels, it can cause damage to plants and horticultural produce. Damage by ethylene results principally from its acceleration of aging. Thus, ethylene accelerates fruit ripening, floral senescence, and leaf abscission (Abeles, 1982a; Abeles et al., 1992). In addition, ethylene can inhibit shoot elongation and promote ovule abortion and young fruit shedding (Abeles et al., 1992). Damage to plants or produce can also occur during harvesting, shipping, and storage. But usually, greenhouse operators and dealers who ship, store and sell fruit, vegetables, flowers, and potted plants have been most affected (Abeles, 1982a, b). Although less likely, damage to outdoor plants can result from

Gas concentrations expressed as volume of ethylene per volume of air (μL L⁻¹ = ppm and nL L⁻¹ = ppb). When authors expressed concentrations as mg m⁻³, data were converted to nL L⁻¹ and μL L⁻¹ and a few of the original expressions shown in parentheses as mg m⁻³ to provide context.
exposure to ethylene. Thus, there is both scientific and practical interest in understanding the responses ethylene can cause and the concentrations of the gas and conditions under which damage can occur.

The development of gas chromatography as an analytical system to measure ethylene concentrations in air (Abeles et al., 1992) has allowed widespread recognition of circumstances where ethylene may be acting as an air pollutant. Thus, one might think that ethylene in air is a simple and well-defined problem. That common assumption is incorrect. There are many reasons that justify a new review on environmental or atmospheric ethylene, including:

1. Ethylene is not a common toxic substance like sulfur dioxide or ozone. As air quality regulations continue to be updated or revised, officials involved need to understand its unique position as both hormone and potential air pollutant.
2. Growers, plant breeders, agronomists, horticulturists, and toxicologists can benefit from a greater understanding of the new information on effects ethylene has on plants and the way it acts.
3. While advances in sensitivity and accuracy of gas chromatography and other newer technologies have allowed detection of ethylene at lower concentrations, understanding of how environmental conditions can alter sensitivity of crops to these lower concentrations of the gas has lagged.
4. Relatively recent progress in understanding the molecular and genetic mechanisms of ethylene action of in plants opens new opportunities to reevaluate data on plant–ethylene interactions.
5. It is desirable to set all air quality standards as carefully as possible. This task is more complex in the case of ethylene since it is produced naturally, making a totally ethylene-free environment impossible.

**ETHYLENE RECEPTORS**

Understanding of ethylene receptors and how they control its actions has advanced rapidly in recent years. Among all plant hormone receptors, those of ethylene are perhaps the best characterized. Ethylene receptor genes were first discovered in the genetic model *Arabidopsis thaliana* (L.) Heynh. using forward genetic signaling on mutagenized plants. Mutations in genes encoding ethylene signaling components were identified by visually detecting plants that would not respond to applied ethylene or plants exhibiting typical symptoms of exposure to ethylene when no ethylene was applied. Some of the mutant genes encoded proteins that are receptors of ethylene, and these genes were isolated and sequenced.

*Arabidopsis thaliana* has five different genes that encode ethylene receptors, including *ETR1*, the first one discovered (Bleecker et al., 1988; Chang et al., 1993; Ecker, 1995). Ethylene receptor genes, similar to those first identified in *A. thaliana*, have been detected in every other plant where a serious effort has been made to identify them (Arora et al., 2006, Chen et al., 2005; Klee, 2004; Kuroda et al., 2004, Ma et al., 2006) and, in the majority of these cases, these other species have also been found to possess multiple receptors. All of these receptors are similar in configuration to *Arabidopsis ETR1*, each containing membrane spanning regions that, in configuration as homodimers, bind copper to provide an ethylene binding site (Rodriguez et al., 1999). The ethylene receptors have been shown to coordinate with proteins encoded by other genes to form an ethylene signal transduction system. The basic components and mechanism of the signal transduction pathway are conserved in agronomically important dicots and monocots (Adams-Phillips et al., 2004; Klee, 2004).

Rodriguez et al. (1999) found that the sequence homology of the ethylene-binding domain in plants corresponds to a functional ethylene-binding domain in the *slrY212* coding sequence from *Synechocystis*. Since an ancestor of *Synechocystis* is thought to have formed the modern chloroplast in higher plants, Rodriguez et al. (1999) suggested that the ancestral *Synechocystis* receptor gene may represent a single origin for all higher plant ethylene receptors. This suggests that a single family of receptors exists, which might determine all of the ethylene responses of plants to ethylene.

Biochemical studies using the *Arabidopsis ETR1* gene expressed in yeast determined a dissociation constant with ethylene of about $2.4 \times 10^{-9}$ M and a dissociation half-life of ethylene with the resultant protein of approximately 12 h (Schaller and Bleecker, 1995). The results indicate that the receptor has a very high affinity for ethylene and releases it slowly once bound. Further tests with other ethylene receptors from *A. thaliana* and tomato (*Solanum lycopersicum*) have demonstrated high-affinity, ethylene-binding activity with parameter values similar to *ETR1* (O’Malley et al., 2005). Plants with any receptor mutated in the ethylene-binding domain show a dominant (gain of function) ethylene phenotype indicating the importance of the individual receptor genes (Klee, 2004, Chen et al., 2005). Conversely, loss of function mutations in any single ethylene receptor gene had little or no effect, consistent with functional overlap within the receptor family (Hua and Meyerowitz, 1998). However, when multiple loss of function receptor mutations are combined, a constitutive ethylene response is observed.

While these descriptions may seem counterintuitive, genetic evidence from mutants shows that the receptors are actually negative regulators (Hua and Meyerowitz, 1998; Chen et al., 2005; Klee, 2002; Wang et al., 2002, and many others). The proposed model for ethylene action is as follows: an empty (unoccupied by ethylene) receptor protein associates with another signaling component from a gene named *CONSTITUTIVE TRIPLE RESPONSE* (*CTR1*) (Clark et al., 1998), and this combination actively prevents subsequent ethylene response steps. When a
receptor protein binds with an ethylene molecule, it can no longer interact with CTR1 protein, which in turn can no longer prevent subsequent steps in the ethylene signal transduction pathway. As a result, ethylene responses occur (Klee, 2004). Mutant receptor genes cannot bind ethylene; thus, they do not terminate their association with CTR1 protein and do not allow the signal transduction chain to cause ethylene responses. This ethylene receptor model predicts:

- An inverse correlation exists between receptor levels and ethylene sensitivity, and
- A threshold for ethylene effects(s) exists.

In other words, when a certain proportion of the receptors are inactivated by binding ethylene, ethylene effects become manifest.

There is evidence for a physical, protein-to-protein association of ETR (ethylene response)-like receptors with CTR (constitutive triple response) protein (Chen et al., 2005; Clark et al., 1998; Wang et al., 2002). This is considered to be additional evidence that the ETR1 family of genes are the only receptors, because mutation of CTR1 produces constitutive ethylene symptoms. In addition, recent studies indicate that rapid increases can occur in receptor protein synthesis in some tissues of some species, thus allowing these tissues to remain insensitive to ethylene (Chen et al., 2005). Collectively, all of the evidence for this model suggests, but admittedly does not prove, that the ETR1 family of genes encodes the only ethylene receptors in plants.

Although the ETR1-like receptors represent the initial step in the entire ethylene response system, this leaves unanswered the question of how this single class of receptors can, in some cases, work more rapidly or at lower concentrations than would be expected. Chen et al. (2005) recently reviewed the phenomenal range of concentrations at which ethylene can promote or inactivate various responses. For example, in dark-grown A. thaliana seedlings, changes in growth rates have been observed at ethylene concentrations as low as 0.2 nL L⁻¹ (0.2 ppb) (Binder et al., 2004a, b); in contrast, fruit ripening involves ethylene production that raises internal concentrations to levels above 100 μL L⁻¹ (100 ppm) (Abeles et al., 1992). Transcription rate changes have been shown to occur over a range of ethylene concentrations from 0.1 to 1000 μL L⁻¹ (Chen and Bleecker, 1995). Thus, the same mechanism (i.e., receptor-mediated expression of genes) appears to be involved at both concentration extremes. Again, these data suggest a mechanism involving a single ethylene receptor.

Chen et al. (2005) suggest that the ETR1-like receptors have a mechanism to recognize and transduce information in response to very subtle changes in levels of receptor occupancy by ethylene. The ability to recognize small changes in ethylene levels suggest a mechanism to regulate responses to environment or development. If these low concentrations could produce damage, then most plants would frequently be exposed and they likely would never complete their life cycles.

One indication that low levels of ethylene regulate adaptive responses comes from exposing A. thaliana seedlings to ethylene in the dark. Such plants exhibit the triple response, which mimics the symptoms of pea (Pisum sativum L.) seedlings growing against a soil crust (Abeles et al., 1992; Goeschl et al., 1966). Growth in the dark upward to contact a soil crust increases ethylene production. Growth subsequently responds to the elevated ethylene levels and elongation is inhibited, the stem diameter increases and, in peas, A. thaliana, and some other species, growth proceeds in the horizontal direction. Light coming through an opportunity located crack in the soil crust will inhibit the production of extra ethylene, and the resulting change in growth rate and direction causes the shoot to return to normal, vertical growth and emerge through the crack (Goeschl et al., 1967). These observations make clear that the triple response is an adaptation response and not really a “damage” response. A number of other responses to ethylene exist that are more likely adaptation responses such as promotion of growth by flooding in deep water rice (Oryza sativa L.) and other frequently flooded species, rapid corolla shedding in ephemeral flowers, and bending of plants toward light or away from shade (Abeles et al., 1992). It is likely that most fast responses to ethylene or responses to relatively low concentrations of the gas are adaptation responses and have evolved to help plants survive stresses that occur in nature or to economize their costs of reproduction.

The other phenomenon, relatively fast responses to ethylene, has also been studied in detail recently. Elongation growth of A. thaliana seedlings is inhibited after only about 15 min exposure to ethylene and full recovery to pretreatment growth rate requires only about 90 min after removal of ethylene (Binder et al., 2004a). The inhibition reaction shows both the fast phase and a slower, steady inhibitory phase.

Various receptor mutations altered the time for recovery to pre-ethylene growth rates. These results represent major evidence that the behavior of plants exposed to ethylene is due to the ETR1-family of genes, and the authors proposed a model by which they could participate in fast reactions (Binder et al., 2004b).

If adaptation responses occur more quickly or in response to lower concentrations of ethylene than those associated with damage responses, how do both types of responses use what are apparently the only receptors available? O’Malley et al. (2005) proposed that the ETR-like receptors may be arranged in and act in clusters in some exceptionally fast responses. Chen et al. (2005) have proposed ethylene action in a range of concentrations and a range of exposure times may result from secondary
modifications of the receptors in various tissues, stages of development, or species. As Klee (2004) points out, there are differences in how ethylene integrates both developmental and environmental cues in A. thaliana, thus we should expect that in other plants.

One final anomaly that affects managing air pollution is the occurrence of substantial changes in sensitivity to ethylene over the lifetime of plants or individual plant organs. Kevany et al. (2007) found that fruit ripening in tomato requires the coordination of both developmental cues and ethylene. Exposure of not-yet-mature tomato fruits to sufficient ethylene drove the level of LeETR4 or LeETR6 down, thus making the fruit more sensitive to ethylene by reducing the overall receptor level. The ethylene-treated fruits then behaved as typical climacteric fruits and ripened in response to ethylene. Since the behavior of ethylene-sensitive flowers is similar to climacteric fruit (Serek et al., 2006), it is likely that climacteric flowers respond to ethylene by reducing their levels of one or more receptors and thus become more sensitive to ethylene. This means that once an accumulation of ethylene exposures from either endogenous or environmental ethylene has lowered the flower’s receptor level, preventing or blocking wilting or shedding will be difficult or unlikely.

The practical application of what is known about the ethylene receptors leads to the following conclusions and recommendations. First, the ethylene receptors have high affinity for the gas but take 12 or more hours to reach saturation. Thus many ethylene responses will require rather long exposures to relatively high levels of the gas. Second, there are some exceptionally fast or exceptionally low concentration responses that are likely to regulate adaptation behavior. The adaptations are mainly responses to environmental stresses and some developmental signals and probably do not initiate major damage to the plant unless the stress approaches a lethal degree. Third, there are some organs, mainly climacteric fruit and flowers, that exhibit a climacteric peak in respiration rate and ethylene production at the start of ripening or senescence (Abeles et al., 1992; Kevany et al., 2007; Serek et al., 2006). These are natural behaviors and are necessary for ripening or senescence to occur. Eventually sensitivity to ethylene increases. This increase in sensitivity is not due to a single brief exposure to ethylene but rather frequent or extended exposures that saturate receptors or overlap the recovery interval needed to prevent the response. Once the organ reaches the ethylene sensitive stage, it has begun to make more ethylene endogenously and protection from external ethylene may become irrelevant.

ACCURACY AND REPRODUCIBILITY IN ETHYLENE RESEARCH

Plants make ethylene via enzymes in their cell solutions, a process called the Yang Cycle (Abeles et al., 1992; Yang and Hoffman, 1984). The gas is not very soluble in water, the major component of the cell solution, and so it moves into the gas phase where it can diffuse from the plant or fruit into the surrounding atmosphere (Abeles et al., 1992). The rate of synthesis varies greatly, and its rate of diffusion out of the plant is a function of the density of the plant or fruit surface, the relation of the surface area to the volume of the tissue, and the concentration gradient from inside to outside. Anything that alters the dynamics of the internal synthesis, escape, or entry by diffusion will affect the internal concentration and thus can alter the growth and development of the plant. In addition, any condition that alters the sensitivity of the plant tissue to ethylene will also alter development of the plant. These variations make it always impossible to judge correctly whether or not the gas may cause damage even with an accurate analysis of the air concentration of ethylene.

Responses to ethylene are influenced by a number of factors including plant species, cultivar, stage of development, physiological activity, temperature, time of day, cultural environment, and presence or absence of stress (Abeles et al., 1992). In addition, whether the subject being considered is an intact plant or a detached plant part influences its response to ethylene. Thus, the concentration that may cause damage will vary depending on the circumstances. Finally, many responses to ethylene only occur at specific times during development, and any potential for damage may be limited to that time frame.

To answer the question accurately about a threshold for damage, researchers have exposed plants to carefully controlled levels of ethylene at specific times in their life cycles. Control of the environment has usually been achieved with plant growth chambers or greenhouses with supplemental environmental control. Such experiments have been conducted by exposing plants grown under artificial conditions to constant concentrations of ethylene. Understanding the way artificial conditions influence plant development and the differential effect ethylene might have on such plants, relative to plants grown in the field, is critical to proper interpretation of research results to derive air quality standards.

Early research on ethylene determined dose–response curves for several effects of ethylene (Abeles, 1973). The threshold for several effects was at 0.01 nL L⁻¹ ethylene, and, based on their data on urban air pollution, Abeles and Heggestad (1973) suggested air quality standards then in force in California should be lowered to 10 nL L⁻¹ for 8 h. Many modern studies adopted <10 nL L⁻¹ for 8 h as a target for standards (Abeles et al., 1992).

This arbitrary target should be rejected now for several reasons. First, when considering a range of species and responses, the dose–response curve is actually a band with threshold effects from 0.01 to 0.1 μL L⁻¹, half maximal effects from 0.1 to 1.0 μL L⁻¹, and saturation from 10 to 100 μL L⁻¹ (Abeles et al., 1992). Not all ethylene responses occur at the lowest concentration nor do such responses occur in all species.
A second reason to question using dose–response curves to set air pollution standards is that they were usually obtained with plants grown in greenhouses or growth chambers. Abeles (1982a, b) recognized that plants from growth rooms or greenhouses were more sensitive to ethylene than those growing in the field. Abeles and Heggestad (1973) found that levels of ethylene in ambient air in the Washington, DC, area caused plant damage in several experiments, but in a later review of this work, Abeles (1982b) cautioned, “However, it is important to recall that those plants (in the 1973 paper) were grown under optimal conditions and that ethylene was the only stress factor in the system. Under normal field conditions, plants would be exposed to internally generated ethylene caused by stresses such as wind, drought, and temperature extremes. Additional stresses such as insects, disease and oxidant air pollution damage would also cause plants to produce ethylene.” Exactly how exposure to stress makes plants less sensitive to atmospheric ethylene is unknown and whether their stress ethylene is involved in all responses is also unknown. However, such growth-inhibited plants produce tissues that are less metabolically active, less succulent, and more woody than tissues of plants grown in artificially controlled conditions, which may contribute to the noted differences in sensitivity to ethylene.

Another limitation inherent in experiments with greenhouse or growth chamber plants is the possible effect of reduced light. Guinn (1976, 1982) found that shade, dim light, or darkness are stresses that promote ethylene production and abscission of cotton (Gossypium hirsutum L.) fruit. Thus moving greenhouse plants into a significantly lower light environment, such as a plant growth chamber, will possibly promote the plant’s sensitivity to applied ethylene.

STUDIES EMPLOYING NATURAL CONDITIONS

Because of the limitations of experiments done in artificial growing conditions as noted above, researchers in the Netherlands sought to evaluate effects of environmental ethylene on crop development and yield in the field (Dueck et al., 2003; Tonneijck et al., 2000). They selected a site with five ethylene-emitting industrial plants and established a monitoring site 1000 m down wind. They measured ambient ethylene levels with an automated monitoring system that had a limit of detection in the 5 nL L⁻¹ range and that recorded data every 10 min for the entire growing season for 10 successive years. They used potato (Solanum tuberosum L.) as the primary test plant (classified as ethylene sensitive by Abeles, 1973; Crocker, 1948; Taylor et al., 1987; and Tonneijck et al., 2000). Yield data were reported for 8 yr, and the hourly average ethylene concentration at the 75 percentile level (average hourly ethylene level expressed as a percentage when ranked from the lowest to the highest) was 28.8 nL L⁻¹ (28.8 ppb), at the 95 percentile level the average hourly ethylene concentration was 111.8 nL L⁻¹, and the seasonal mean was 24.9 nL L⁻¹. All of these values were for intermittent exposures, which exceed the arbitrary threshold for ethylene effects of 10 nL L⁻¹ adapted from dose–response curves for continuous exposures by Abeles and Heggestad (1973). The average number of hourly observations for the years in question was 2723; therefore, for an average of about 680 h per growing season the ethylene level exceeded 28.7 nL L⁻¹ (the 75 percentile level) and for over 136 h per growing season the ethylene level equaled or exceeded 112.2 nL L⁻¹ (the 95 percentile level). When the yield data for all 8 yr were combined, statistical tests revealed no significant differences (p < 0.05) in tuber yield between the experimental site and the control site 5 km away from the wind direction from the industrial sources of ethylene. Indeed, tuber yield was highest in 1982, the year the growing season mean ethylene concentration was highest (53.5 nL L⁻³) and yield was lowest in 1989, the year with the next to lowest growing season mean ethylene level (14.8 nL L⁻¹). The results of this study indicate that the 10 nL L⁻¹ (10 ppb) threshold is far below a level that will cause air pollution damage in potatoes, if exposures are intermittent and not at a constant concentration, neither of which are typical conditions of exposures used to generate dose–response curves (Abeles, 1973; Abeles et al., 1992).

To broaden their study, for 7 yr Tonneijck et al. (2003) placed potted petunias (Petunia nctaginiflora Jugg. cv. White Joy) and marigolds (Ragetas erecta L.) in pots at the same location as the study with potatoes growing in soil (Tonneijck et al., 2000). Petunia is classified as ethylene sensitive and recommended as a screening plant for ethylene effects (Posthumus, 1983). Additionally, marigold is known to exhibit both flower bud abscission and leaf epinasty in response to ethylene (Crocker, 1948). For 2 yr pots were located on a grid extending from the center of the five ethylene-emitting industrial facilities further on downwind toward the ethylene monitoring station used in the potato experiment (Tonneijck et al., 2000). For those 2 yr, data for ambient ethylene concentrations were shared for the two experiments (Tonneijck et al., 2000, 2003). Damage to flowers and plants of petunias and marigolds did not occur at any time at the monitoring station (1000 m downwind), and it diminished markedly from the center of the release site until disappearing at 460 m downwind for petunia and at 400 m downwind for marigold. (Tonneijck et al., 2003). Plants exposed near the monitoring station had more flowers than the unexposed control while their growth was normal.

In the above experiments, the levels of ethylene varied markedly and frequently (Tonneijck et al., 2000, 2003). To achieve a more predictable exposure at levels well above those predicted to cause damage based on dose–response curves with tender plants, an experiment was conducted
using 18 open top chambers (OTC) partially enclosing sections of rows of potatoes growing in a field without ambient ethylene pollution (Dueck et al., 2003). The OTC were equipped to emit ethylene near the soil surface that could then escape at the top of the chambers. Design of the OTC maintained air exchange at approximately 3.6 times min−1. Potato plants were exposed to 200, 400, or 800 nL L−1 ethylene for 3 h twice a week or to 200 or 400 nL L−1 for 3 h three times a week. Treatments in this experiment continued for an entire growing season, yet none of them caused yield reductions. Occasionally some of the treatments reduced flower number and/or promoted leaf epinasty.

The investigations in the Netherlands illustrated that in the field ethylene-sensitive plants did not show damage unless concentrations of the gas were significantly higher than the 10 nL L−1 threshold proposed in earlier studies (Abeles and Heggestad, 1973). In contrast to the relative insensitivity of plants to ethylene in these outdoor studies, Klassen and Bugbee (2002) observed significant damage to plants exposed continuously, under controlled laboratory conditions, to relatively low levels of ethylene in small containers. Ethylene was applied 24 h per day at constant levels in the containers, which were in growth chambers from 7 d after emergence until harvest. Yield was reduced by 36% in USU-Apogee wheat (Triticum aestivum L.) and 63% in Super Dwarf rice when exposed to 50 nL L−1 ethylene, and both species were virtually sterile when continuously exposed to 1000 nL L−1. However, the identical 1000 nL L−1 ethylene concentration only slightly reduced yield of wheat if exposure was terminated before anthesis. In greenhouse experiments, which employed two cultivars of wheat (USU-Apogee and Super Dwarf) but not Super Dwarf rice, higher light intensities, slightly larger flow-through containers, and 24 h photoperiods, 50 nL L−1 ethylene reduced yield of Super Dwarf wheat but not yield of USU-Apogee wheat. Exposures were again from 7 d after emergence until harvest. The inconsistency in response of USU-Apogee wheat to ethylene in the growth chamber versus the greenhouse experiments suggested to the authors an interaction between the ethylene treatment and environmental conditions in that cultivar.

Klassen and Bugbee (2004) also reported reduced growth of several vegetable species when germinated and grown in constant levels of 25 to 250 nL L−1 ethylene for undefined durations in small, flow-through chambers. The experiments at Utah State University (Logan, UT) by Klassen and Bugbee (2002, 2004) were unusual in that the background ethylene levels were consistently below their detection limit (5 nL L−1), while the background levels in the Netherlands were detectable (above 5 nL L−1) and generally below 10 nL L−1 (Tonneijck et al., 2000, 2003). The differences are consistent with the possibility that exposure to stress from growth outdoors can reduce the sensitivity of plants to ethylene in some situations (Abeles 1982a, b).
experiments with variable environmental conditions. They conducted tests using greenhouse plants exposed to ethylene in especially modified and equipped commercial plant growth chambers (Li and Archambault, 1998). Before the detailed tests, Archambault et al. (2006) (dates on the Canadian references are not in the order in which the work was done) screened several cultivars of local crop species for sensitivity to ethylene at various developmental stages. They used ethephon (2-chloro-ethylphosphonic acid) as the test source of ethylene, and they concluded that barley (Hordeum vulgare L.), wheat, and canola (Brassica napus L.) were most sensitive in the late vegetative and early reproductive stages with sensitivity defined as reduction in yield and seed quality. For field pea, ethylene (ethephon) did not reduce yield but delayed maturity and increased the number of pods. The results allowed the investigators to conduct their study with a range of species important to the province and to treat them with ethylene gas at a time when they were most sensitive to damage.

The plants for subsequent studies were grown in standard greenhouses before and after exposure to ethylene in growth chambers (Archambault and Li, 2001). The ethylene exposures were done in commercial plant growth chambers (Conviron E15 [Conviron, Winnipeg, MB, Canada] with 1.13 × 1.83 × 0.73 m height × length × width and 1.5 m² floor area) (Li and Archambault, 1998). Ethylene was delivered and monitored by a sophisticated control system. A remote, centrally controlled watering and fertilizing system functioned without opening or closing the exposure chambers. All of the parameters measured indicated that the exposure system was accurate and dependable. Conditions maintained in the exposure chambers were similar to those in the greenhouses; photoperiod of 16 h light, 8 h dark, and photon intensities at midcanopy were maintained to those in the greenhouses; photoperiod of 16 h light, 8 h dark, and photon intensities at midcanopy were maintained approximately 23°C in the day time and 15°C at night.

In short term, continuous exposures to ethylene lasting up to 12 h and concentrations up to 1200 nL L⁻¹, no significant effects on vegetative or reproductive characteristics were observed for barley, field pea, or canola (Archambault and Li, 2001). Longer term, continuous exposures to ethylene were conducted (Archambault and Li, 2001), and treatments were always centered on the most sensitive stage determined previously (Archambault et al., 2006). In these experiments, several vegetative and reproductive effects were observed. All yield reductions are expressed relative to controls. barley cv. Harrington was more sensitive to ethylene exposure than either field pea cv. Carrera or canola cv. Quantum. While barley showed no effect of ethylene on vegetative growth or development at concentrations up to 400 nL L⁻¹ for 26 d from flag leaf emerging to early anthesis stage, it did show a strong reduction in reproductive development in the same experiment. Number of heads, weight of heads, and seed number per pot decreased by 89, 98, and 100%, respectively. At 30 and 50 nL L⁻¹ ethylene, seed yields decreased by 63 and 72%, respectively. Decreased seed yields were due to both lower seed numbers and decreased seed size and were observed only when longer term exposure occurred during reproductive development. With both barley and field pea, ethylene fumigation increased root biomass. The effect of duration of treatment on yield was tested. At 50 nL L⁻¹, seed yield of barley was decreased after only 6 d exposure centered around the spike emerging stage. The number of seed was decreased by 27% in 3 d and by 86% in 26 d. Although seed size was reduced, the major effect was the reduction of seed number. In contrast to barley, for field pea, ethylene exposures of 50 nL L⁻¹ for up to 26 d caused no reduction of either yield or seed quality. In another grain crop, Reid and Watson (1982) found that yield of oat (Avena sativa L.) was reduced by 84 and 99% when plants were exposed to ethylene for 100 d at 70 or 150 nL L⁻¹, respectively.

Long-term, continuous ethylene treatment of field pea slowed maturity, and results were different when compared at maturity of control plants or compared later when the ethylene-treated plants reached maturity. At the earlier harvest, seed yields were reduced up to 73% at 400 nL L⁻¹ ethylene, but later, when the fumigated plants reached maturity, there was no reduction in yield compared to the controls harvested earlier. The effect on yield was almost completely on seed size; no difference was noted in seed number.

Canola responded to long-term ethylene rather differently than barley and field pea. Height of canola was reduced 40% by 400 nL L⁻¹ ethylene for 31 d, while no effect was noted with barley and height of field pea was increased. Seed yields of canola were reduced up to 77% by 400 nL L⁻¹ ethylene, due mostly to decreased seed numbers. Similar to barley, canola did not recover yield loss after long term exposures to ethylene, and recovery by peas is assumed due to their continued flowering after exposure.

To test the effect of exposures at different times of day, barley plants were exposed to 200 nL L⁻¹ ethylene for one of four 6-h periods of the day for 30 d, centered on the spike-emerging stage (Archambault and Li, 2001). Number of heads, weight of heads, and seed yield were reduced only by treatments in the middle of the light period (1000 h to 1600 h). No reduction in seed size occurred, in contrast with results for other treatment regimes. Fourteen days at 100 nL L⁻¹ ethylene applied continuously caused 100% yield loss, suggesting to the authors that the intermittent pattern of the 6-h exposures allowed time for recovery from reduction of seed yield (Archambault and Li, 2001). This is consistent with dissociation and escape of some of the ethylene by diffusion between treatment periods (see Ethylene Receptors, previously).

Based on their results, Archambault and Li (2001) concluded that the interim 6-h Alberta Air Quality Guideline of 104 nL L⁻¹ (120 mg m⁻³) conservatively protected barley,
canola, and field pea from yield reduction due to short-term ethylene exposure. The interim 30-d Alberta Air Quality Ethylene guideline (44 nL L⁻¹) appeared to protect against yield reductions in canola and field pea; however, yield reductions may occur in barley at levels below that guideline. The potential for these yield effects is limited to exposure during the most sensitive stage of development, which was the 26-d period from flag leaf emerging to early anthesis. Damage to grain crops due to exposure to ethylene during or near anthesis has now been demonstrated in artificial growth conditions in four major grain crops: oat (Reid and Watson, 1982), barley (Archambault and Li, 2001), and wheat and rice (Klassen and Bugbee, 2002).

One must question whether the plants used in the Alberta study were somewhat more sensitive to ethylene than if they had been grown outdoors since greenhouse grown plants are subject to less environmental stimuli, such as wind movement, that may elicit ethylene responses or some as yet undefined hardening. It should also be noted that for barley, in tests for concentration effects, exposures to ethylene continued 24 h d⁻¹ for 26 d and so plants were in the growth chamber with reduced light and less temperature variability than would occur even in the greenhouse for that extended period. Thus, the most reasonable estimate is that these plants were somewhat more sensitive to ethylene than similar plants grown in the field. It is very likely that for the species selected in the Alberta study, the use of arbitrary standards based on early dose–response curves showing responses at 10 nL L⁻¹ (Abeles and Heggestad, 1973; Abeles, 1973) is not warranted, a conclusion made by Abeles (1982b) earlier.

Flowers
Among the earliest observations of ethylene pollution effects were responses of flowers. For instance, the phenomenon of carnation flowers “going to sleep” initiated interest in the biology of ethylene in the United States (Cocker and Knight, 1908). Later, workers on the East Coast and the West Coast noted damage to orchid flowers (Cattleya trianae and several other species), apparently by low levels of ethylene (Davidson, 1949; Darley et al., 1963). In the 1950s, Hall et al. (1957) observed ethylene damage to cotton growing in fields near a polyethylene manufacturing facility in Texas. Hall (1952) and Hall et al. (1957) noted that abscission of preflowering cotton buds, flowers, and young fruit was much more sensitive to ethylene than leaf abscission and other visible effects. These studies all occurred in the pre–gas chromatography era and thus the quantitative data reported may be inaccurate; nevertheless, there was a general impression that flowers are particularly sensitive to ethylene.

Woltering (1986) pointed out that European horticultural growers apparently unknowingly selected for commercial production plants that are not very ethylene sensitive. For example, when over 50 species of ornamental potted plants were tested for ethylene sensitivity (shedding of leaves, flower buds, and flowers), the 25 economically most important species (most commonly produced, shipped, and sold) were much more commonly ethylene insensitive (88.8%) versus the 25 species less commonly grown that were less ethylene insensitive (11.2%). The implication is that as the modern horticulture industry has developed, growers and dealers have selected species to grow and sell that do not demonstrate the tendency to shed leaves, flower buds, flowers, or fruit or exhibit flower wilting or leaf epinasty. These same species were less sensitive to ethylene to which they might be exposed during production, transport, marketing, and utilization in the consumer’s environment. Thus one way to minimize air pollution damage is to avoid using the most ethylene-sensitive species. While this approach alone is not a satisfactory answer to air quality problems, it does point to the use of genetic selection and use of mutant genes in breeding ethylene-insensitive, ornamental flowering plants as one useful approach to environmental problems.

Petunias have already been transformed with the dominant mutant A. thaliana ethylene receptor gene etr1-1 to inhibit ethylene responsiveness, and while it did increase floral longevity, its decrease in ethylene sensitivity also caused transformed plants to have significantly less adventitious root formation (Wilkinson et al., 1997; Clark, 2004; Clark et al., 1999). The increase in floral longevity was outweighed by the reduced root development, showing the complexity of genetic solutions. Flowers abscise and petals shed or wilt in both monocots and dicots (Woltering, 1987; van Doorn and Stead, 1997; van Doorn, 2002a). While these events are natural, they can also be promoted by ethylene, anthesis, and normal development. In most cases, species showing natural flower and/or petal abscission are more sensitive to ethylene, thus illustrating the difficulty of protecting such plants from intermittent air pollution (Klee and Clark, 2004; van Doorn, 2002b). Flowers that synthesize ethylene are usually sensitive to the gas, and this ethylene is usually involved in aiding flowers to discard their no longer needed parts after fertilization occurs (Klee and Clark, 2004).

Pelargonium spp. (geranium) exhibit remarkable developmental differences in sensitivity to ethylene (Evensen, 1991; Clark et al., 1997). In freshly opened flowers, petals will not abscise after exposure to 100 μL L⁻¹ ethylene, while after only two or three additional days, ethylene concentrations as low as 0.5 μL L⁻¹ will cause complete and rapid petal abscission (Evensen, 1991). A short burst of ethylene produced within minutes of pollination causes complete petal abscission within a couple of hours (Evensen, 1991; Clark et al., 1997). These rapid responses to relatively low levels of ethylene can be explained by existing information and models. For example, if the petals have been exposed to increasing endogenous ethylene, thus inactivating
ethylene receptors, subsequent exposure to ethylene would be expected to promote rapid abscission.

The ultrasensitivity of flowers of geranium contrast with plants such as petunia, carnation (Dianthus caryophyllus L.), and orchids, which gradually and progressively become more sensitive to ethylene as they age and/or approach the stage of development that is optimal for pollination (Klee and Clark, 2004). Thus, increasing sensitivity to ethylene and increasing ability to synthesize ethylene go hand in hand and make prevention of damage from environmental ethylene increasingly difficult up to the time that senescence will occur exclusively due to natural developmental causes. van Doorn (2002b) conducted a survey to test excised flowers of over 300 species in over 50 different families for their sensitivity to ethylene. The exposure was to 3 μL L⁻¹ ethylene for 24 h. While more species exhibited intermediate, high, or very high sensitivity (64%), a substantial percentage (36%) were insensitive or low in sensitivity to ethylene. This suggests that sensitivity to ethylene is not a fatal problem for floriculture but one that needs continued attention, especially where production occurs in or near urban or industrial areas.

van Doom’s data (2002b) and other comparisons in this section suggest two conclusions concerning the potential for damage to flowers from ethylene in air. First, not all species are susceptible and second, at least some species present a “moving target.” The “moving target” expression indicates that as many flowers age they become more sensitive to ethylene and they also produce more ethylene. Preventing their exposure to environmental ethylene will eventually not delay senescence or abscission.

Because flowers and fruits make ethylene, often in an autocatalytic fashion, there is a growing interest in treatments that can be applied to block or delay ethylene-mediated abscission of flowers and flower parts and wilting of the same (Blankenship and Dole, 2003; Serek et al., 2006; Woltering, 1987). Chemicals such as AOA (α-aminooxyacetic acid) and AVG (aminoethoxyvinylglycine) reduce ethylene biosynthesis and reduce or delay floral senescence, but their commercial development is limited because they do not alter sensitivity to external ethylene (Klee and Clark, 2004). Silver ion, discovered in studies on ethylene action (Beyer, 1976a, b), was eventually developed in the form of silverthiosulfate (STS) as a treatment applied by spray to protect flowers from ethylene (Veen, 1979, 1987). It was widely used and, for a period, was required on some species to qualify them to be sold in certain flower markets (Abeles et al., 1992). However, environmental concerns of heavy metal toxicity eventually limited or eliminated its use (Abeles et al., 1992). More recently, 1-methylenecyclopropene (1-MCP) was discovered to be an effective ethylene action inhibitor (Sisler and Blankenship, 1996). 1-Methylenecyclopropene was initially applied as a gas and the duration of its effectiveness was limited, especially in species with rapid turnover of ethylene receptors. More recently, sprayable liquid formulations of 1-MCP have been developed and are being tested as a means to protect plants from ethylene (Blankenship and Dole, 2003).

CONCLUSIONS
Gas chromatography has made it possible for researchers to quantify very low levels of ethylene and, combined with sophisticated, flowing-air fumigation chambers, to illustrate responses to or damage from accurately determined levels of ethylene. In addition, there is evidence for the presence of ethylene in some urban and industrial environments at levels that may damage plants in artificial environments. In recent years researchers have been able to expose plants growing outdoors to ethylene at concentrations that had been judged to be damaging using artificial growth environments. Surprisingly, these outdoor plants and others growing in commercial plant growth chambers were less sensitive to ethylene than those treated under more artificial conditions. These results raise questions as to whether proposed air pollution standards in some jurisdictions are too strict. The lower standards are set, the higher costs are for monitoring and enforcement. However, the higher standards are set, the greater are the chances for damages to plants and produce. Additionally, regulators must decide whether to protect against yield losses or against all visible cosmetic effects, a political decision. Universal standards are not advisable because of the great differences in plants and climate from area to area. Research reviewed here from the Netherlands and Alberta, Canada, reflect the need to set standards in a manner that protects vegetation from undesirable effects of excessive levels of ambient ethylene with recognition of variations in sensitivity among and within species and among various climatic conditions. Details will vary from region to region.

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