Ethylene Action in Plants

Nafees A. Khan (Ed.)

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With 31 Figures, 1 in Color, and 6 Tables

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Foreword

The discovery of the plant hormone ethylene was stunning—ethylene is a simple gas! Our expanding knowledge of the multiplicity of ethylene's roles in plant development, physiology, and metabolism makes the study of this plant hormone increasingly compelling. Elucidation of the genetic regulation of ethylene biosynthesis, characterization of ethylene receptors and analysis of the pathway of ethylene signal transduction, coupled with the identification of components in the cascade and target genes, have provided insight into how this simple molecule can drive such a diversity of divergent processes. These scientific advances will lead to new technologies that will further enable researchers to harness the powers of ethylene for the benefit of agriculture.

In *Ethylene Action in Plants*, classic and emerging roles of ethylene in plant developmental processes are integrated through recent advances characterizing ethylene receptors, promoters and antagonists, and biological and environmental factors that mediate ethylene responses. The book's editor, Dr. Nafees Khan, Aligarh Muslim University, Aligarh, India, an expert on ethylene with an impressive number of publications on the interactions between ethylene, photosynthesis, and growth of *Brassica* spp, brought together a highly qualified group of international experts to provide state-of-the-art information. To simply list the topics included does not do justice to the book's contents, as the articles are not just a compilation of the literature relevant to the topic. The authors have synthesized traditional ethylene research with recent novel discoveries to provide both the means for understanding what have previously been considered conflicting results and answers to previously unanswered questions. The book is designed to provide the reader with the details of major strides in ethylene research, including introduction to new areas of research. I offer the following as a brief glimpse into the pages of *Ethylene Action in Plants*.

Ethylene has long been known as the "ripening hormone", but in recent years progress in identifying ethylene receptors in responsive cells and components of the ethylene signal transduction pathway, including transcription factors and target genes controlling ripening-related processes in fruit and vegetables, has been dramatic. In *Ethylene Action in Plants*, advances in the genetic regulation of ripening are detailed in relation to the role played by other hormones and with goal of delineating the differences

among developmentally regulated, ethylene early responsive and ethylene late responsive genes. Knowledge of the molecular basis for fruit ripening will undoubtedly result in improved post-harvest longevity, and increased aesthetic and nutritional quality. In recent years, the investigation of promoters and antagonists to the binding of ethylene with its receptor has lead to the identification of numerous compounds that mediate the interaction. The binding and activity of these compounds are described, along with their potential benefit to basic research and agricultural. It is the hope that the specifics given in the book might lead its readers to discover additional regulatory compounds of value. Whereas the role of ethylene in expansion growth is well known, its effects on biomass accumulation remain understudied, particularly in relation to plants growing under limiting environmental conditions, where ethylene should logically be a factor in the growth response of the plant. In *Ethylene Action in Plants*, the effects of endogenous and exogenous ethylene on growth parameters in optimal and stressful environments are unraveled. Enhanced ethylene production is a common plant response to numerous stresses, but recent evidence that ethylene perception and signal transduction are also affected by stress has lead to the new insight into ethylene sensitivity during stress and stress adaptation presented in the book. Leaf senescence, the last phase of leaf development, is a genetically programmed process. Ethylene plays a key role among leaf senescence inducers. In *Ethylene Action in Plants*, the sequence of events resulting in leaf senescence is described in detail in relation to the physiological effects of ethylene on the process and in light of new research on the modification of ethylene effects by biological and environmental factors that act as promoters and antagonists of ethylene. At best, the role of ethylene in adventitious root development is confusing due the variable responses to ethylene reported in the literature. These variable responses are discussed with the outcome being a better understanding of the basis for the variability and resolution of the conflict. Ethylene also mediates nodulation responses of roots. Comparison of different rhizobium-legume symbioses and their respective nodulation processes provides clarification of contrasting requirements for ethylene in the different bacterial invasion mechanisms involved in nodulation and of the role of ethylene in further nodule development. Another controversial aspect of ethylene physiology discussed in the book is ethylene's role in regulating stem gravitropic curvature. Here, historic evidence and traditional methods are critically evaluated in light of recent advances in the field. The interactions between ethylene and photosynthesis and growth are complex due to modulation of the effects of ethylene by many factors. Evidence is provided to support the involvement of 1-aminocyclopropane carboxylic acid synthase, the rate-limiting enzyme in the synthesis of ethylene, as a common factor in the control of photosynthesis and growth. *Ethylene Action in Plants* integrates results from physiology, biochemistry, and molecular biology research.

Readers of *Ethylene Action in Plants* will gain an appreciation for how significantly our understanding of ethylene action has advanced in recent years

and for current efforts by researchers to answer those questions that remain unanswered and to pose new questions. The book will expand the knowledge base and stimulate the thinking of plant biology graduate students and researchers, be they botanists, ecologists, horticulturists, agronomists, physiologists, molecular biologists, or genetic engineers.

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Preface

Ethylene, the simplest plant growth regulator, has been recognized to control many physiological processes in plants, including fruit ripening, abscission, senescence, and responses of biotic and abiotic stresses. Since the time of the Egyptians, ethylene has been used to stimulate the ripening of figs and the Chinese used it to enhance the ripening of pears. The phenomenon of 'triple response' induced by ethylene was discovered in 1864 when it was noticed that gas leaks from street lamps caused stunting of growth, twisting of plants, and abnormal thickening of stems. It was Neljubow in 1901 who discovered that the active principle in illuminating gas was ethylene; thus, he is credited with the discovery that ethylene is a biologically active gas. Later, in 1934, Gane provided chemical proof that plants produce ethylene. It has now been recognized that ethylene is produced in all higher plants. Thus, with the recognition of the presence of ethylene in plants, the stage was set to investigate the ethylene action in plants as signal molecules. The action of ethylene as a signal molecule depends on its tissue concentration and the ability of the cells to monitor the changing concentrations of ethylene and transduce this information into physiological responses. The effectiveness of ethylene requires high-affinity receptors. It is bound by a membrane-localized receptor. The N-terminal domain of the receptor protein is responsible for binding of ethylene. Components of the ethylene signal transduction pathway have been identified by genetic studies in *Arabidopsis*. Transduction of the ethylene signal is thought to be achieved through a series of phosphorylations that are carried out by a cascade of protein kinases similar to the mitogen-activated protein kinase pathway. Genetic manipulation of the genes responsible for the ethylene signal transduction pathway will provide agriculture with new tools to prevent or modify ethylene responsible in a variety of plants.

The intent of this book is not to cover all the aspects of ethylene biology but to summarize and provide an update on our current understanding on mechanism and regulation of ethylene action. I extend my gratitude to all those who have contributed in making this book possible. Simultaneously, I would like to apologize unreservedly for any mistakes or failure to acknowledge fully. Finally, I thank my family for their continued support and encouragement throughout the work.

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Contents

1 Interaction of Ethylene and Other Compounds with the Ethylene Receptor: Agonists and Antagonists

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1.1 Introduction

Although ethylene has long been recognized as a plant hormone, it is only recently that the ethylene receptor has been subjected to detailed study. Most reviews on ethylene signal transduction do not discuss much about ethylene interaction with the ethylene receptor except to mention that ethylene does bind to the receptor. This review will concentrate on the interaction of ethylene, ethylene agonists, and antagonists with the receptor. It is important that we identify the factors that determine compound binding and activity whether the compound is an ethylene agonist or an ethylene antagonist. It is important that findings from past work be noted in concordance with newfound results that contribute to our knowledge of the many compounds known to bind to the receptor. In recent years, the number and type of compounds that interact with the receptor has been expanded considerably. Some of these compounds appear to be useful both for basic research and for practical purposes. Many more may be discovered. It is the intent here to present some of what is known about both ethylene antagonists and agonists that have been found with the hope that the information will help lead to other compounds.

1.2 Ethylene and Agonists

1.2.1 Discovery of Ethylene Action and Some Important Lessons from the Past

Ethylene is one of the five original basic plant hormones. Many of the responses caused by ethylene were observed before it was known that it was the cause of the response (Abeles et al. 1992). In 1901, Neljubov reported that

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Ethylene Action in Plants
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ethylene caused a triple response in etiolated pea seedlings: i.e., epicotyl thickening, growth retardation, and horizontal growth of the epicotyl. It was soon recognized that ethylene was not alone in causing a triple response in plants. Soon it was known that propylene, acetylene, and carbon monoxide were ethylene agonists also giving a triple response in pea. In 1967, Burg and Burg identified several other alkenes and alkene-related compounds that were active. Isocyanides were added to the list of ethylene agonists 10 years later (Sisler 1977). These were important clues as to how ethylene may act. Now a great number of plant responses have been shown to be regulated by ethylene. Ethylene, which is produced by almost all plants, mediates a wide range of different plant responses and developmental steps. Ethylene plays an active role in seed germination, tissue differentiation, formation of root and shoot primordia, root elongation, lateral bud development, flowering initiation, anthocyanin synthesis, flower opening and senescence, pollination, fruit degreening and ripening, the production of volatile organic compounds responsible for aroma formation in fruits, leaf and fruit abscission, the response of plants to both biotic and abiotic stress, and plant-microbial interactions that are important for plant's growth and survival (Abeles et al. 1992; Grichko and Glick 2001a). Agricultural and horticultural loss is high due to ethylene-accelerated post-harvest ripening and deterioration of perishable commodities.

1.2.2 Molecular Requirements

In 1967, Burg and Burg published a paper on the molecular requirements for ethylene action in plants. Applying techniques used in enzyme kinetics, they compared a number of active compounds for their ability to give an ethylene agonistic response in peas. Using a gas chromatographic technique, they also compared the ability of the same compounds to bind to silver ions. They reported the binding of the compounds to silver ions to be in the same order as their ability to inhibit pea seedling growth. Burg and Burg (1967) then proposed that there was a metal in the supposed ethylene receptor. This was an important step toward understanding the way ethylene acts to bring about a response in plants. That ethylene binds to certain metals was not new. It had been known since 1827 that ethylene formed a complex with platinum and there was much chemical literature available on metal complexes of ethylene and other olefins, but the report by Burg and Burg (1967) was the first report extending this concept to plant responses. Consequently, there were several early attempts and suggestions to explain the mechanism of ethylene activity. Did ethylene act by being oxidized? Did ethylene act by producing some essential component as in an enzymatic reaction, or did ethylene serve to turn on a signal transduction pathway? Experimental evidence has favored a signal transduction pathway and this has been the focus of much recent work.

Some early work focused on the putative metal involved in ethylene action. Based on some deficiency experiments, Burg and Burg (1967) found that only zinc deficiency seemed to alter ethylene sensitivity in plants. For ethylene oxidation, copper seemed more likely as the metal. The reversible binding of ethylene to Cu(I) was well known (Cotton and Wilkinson 1980) and it seemed a likely prospect for being the metal involved (Sisler 1976, 1977). To support the proposed role of monovalent copper in the ethylene binding in plants, complexes of Cu(I) with imidazole-like ligands were synthesized (Thompson et al. 1983; Thompson and Whitney 1984; Thompson and Swiatek 1985). The complexes were the rather stable Cu(I) adducts with ethylene and its agonists and exhibited either a trigonal-planar geometry or a distorted tetrahedral structure. In a membrane environment, ligands bound to a metal ion may considerably alter its properties and the properties of its complex with ethylene, and there is a possibility that other metals might be involved in ethylene binding *in situ*. Rodriguez et al. (1999) did include other metals in an *in vitro* study where ethylene receptor gene *ETR1* from *Arabidopsis* was cloned in yeast. Only Cu(II) and Ag(I) significantly increased ethylene binding. Supplying ions such as Fe(II), Co(II), Ni(II), or Zn(II) did not increase ethylene binding. In the 2-D model of an ethylene receptor, which was developed based on these experiments, the transmembrane, hydrophobic ethylene-binding domain contained one Cu(I) ion per protein dimer, and coordinating amino acids were thought to be Cys65 and His69 (Rodriguez et al. 1999). The ethylene receptor has been suggested to contain either one or two Cu(I) ions per dimer (Hirayama et al. 1999; Pirrung 1999; Klee 2002; Taiz and Zeiger 2002; Weiler 2003). The stoichiometry gives little clue as to the structure. The coordination number of Cu(I) ions can be anywhere from two to six, and it is possible that Cu(I) forms a tetrahedral complex with both Cys65 and His69 (Pirrung 1999). A sulfur-ligated Cu(I)-ethylene complex exhibits very weak metal-ligand bonding interactions (Hirsh et al. 2001), and it is also possible that each cysteine residue is not a coordinating ligand. Cysteine residues instead may form disulfide bond *in situ*, and histidine residues and water may serve as ligands. Ethylene is likely to displace a weak ligand, and water is one of the most suitable candidates for this role. Displacement of water by ethylene followed by expelling of water molecule(s) from the hydrophobic domain is likely to result in the formation of a stable complex. An experiment in which a specific metal is shown to function *in situ* in ethylene perception has not yet been reported and is needed, but much recent evidence has favored copper as the metal involved in the receptor. Hirayama et al. (1999) restored antagonistic activity to an *Arabidopsis ran1* mutant, which gave an agonistic response with *trans*-cyclooctene (TCO), by either cloning a Cu(I) transporter into it or by supplying Cu(II) ions. This essentially confirms that copper can function in the receptor. The fact that *ran1* loss-of-function mutants were responsive to both ethylene and *trans*-cyclooctene is rather fascinating. Because 1-methylcyclopropene (1-MCP), a potent ethylene antagonist, also appeared to function normally, a metal

must have been present in the receptor. Was that metal copper? *trans*-Cyclooctene was not included in the *in vitro* study of Rodriguez et al. (1999), and it is not known if the ethylene receptor associated with a different metal binds alkenes other than ethylene. Unusual behavior of the *ran1* mutant might be a result of either alteration of ethylene receptor conformation, decrease in ligand specificity, or the stability of receptors (Hirayama et al. 1999; Woeste and Kieber 2000). It can also be a result of irreversible disruption of altered ethylene receptors by *trans*-cyclooctene, enhanced sensitivity or insertion of different metal into some ethylene receptors under the conditions of a severe Cu(I) ion deficiency.

Some data suggest that binding of ethylene to the receptor may result in a structural rearrangement of the receptor, which can serve as an initial event in a signal transduction pathway. The role of histidine kinase activity of the ethylene receptor subfamily I is proven to be rather complex (Wang et al. 2003; Qu and Schaller 2004). It was shown that the ethylene receptor directly interacts with the downstream negative regulator CTR1 (Clark et al. 1998), and kinase activity of ETR1 is not required for its interaction with CTR1 (Gao et al. 2003).

There are still many questions about how ethylene acts. The exact structure of the ethylene-binding domain of the ethylene-receptor family is still unknown. The 3-D structure may be determined soon following a high-level expression of ETR1 in *E. coli* (Voet-van-Vormizeele and Groth 2003) and this may greatly facilitate the process of selection of the best candidates from the pool of synthetic compounds and phytochemicals and make it easier to predict anti-ethylene potency of their derivatives.

1.2.3 Ethylene Binding

Another important step in understanding the action of ethylene was the development of methods of measuring ethylene binding in plants. Using ^{14}C-ethylene, the rate of ethylene binding and the rate of ethylene release could be measured in plant tissue (Jerie et al. 1979; Sisler 1979). Using this technique, it could be shown that in vegetative tissue, there appeared to be a major component that bound and released ethylene rapidly. The time-radiolabeled ethylene remained bound to the major component varied in different plants. In most plants, the $t_{1/2}$ was about 10 min. However, in tomato leaflets it was only 2 min. The rapid component correlates well with the data of Warner and Leopold (1971) for response times by pea plants to ethylene. The value for K_d as determined by a Scatchard plot correlated well with the value for K_m as determined by a Lineweaver-Burk plot (Sisler 1979). There usually also was a small amount that was released with a much longer half-time. In some seeds, there were large amounts of ethylene, which remained bound for long periods of time. Because there is no known

function for ethylene in these seeds, this probably represents binding to a storage component.

In measuring ethylene action, pea plants responded to ethylene in just 10 min and recovered with $t_{1/2}$ of about 18 min after its withdrawal (Warner and Leopold 1971). In *Arabidopsis* hypocotyls of etiolated seedlings, there were two phases of growth inhibition by ethylene, a rapid phase followed by a prolonged slower phase. Full recovery occurs about 90 min after ethylene removal (Binder et al. 2004a). The recovery time was significantly smaller than the time of ethylene dissociation from ETR1 receptors expressed in yeast (Schaller and Bleecker 1995; Binder et al. 2004a). The inhibition appears to be a complex process (Binder et al. 2004b). In ethylene binding studies, the shortest value of $t_{1/2}$ for ^{14}C ethylene diffusion from the binding site measured *in vivo* was 2 min (Sisler 1982).

In vitro, the short-lived component is absent; in extracts of mung bean sprouts, $t_{1/2}$ of 1 h and $t_{1/2}$ of 50 h were measured (Sisler 1990). In a cell-free system from cotyledons of *Phaseolus vulgaris*, $t_{1/2}$ was about 10.5 h (Bengochea et al. 1980). In yeast expressing ETR1 at a level of about 4.0×10^{-8} M, $t_{1/2}$ was 12.5 h (Schaller and Bleecker 1995). Based on K_d and $t_{1/2}$ (Sisler 1991), one can estimate that the rate constant of ethylene binding to the receptor is about 5×10^{7} M^{-1} s^{-1} for the short-lived component and 2×10^{5} M^{-1} s^{-1} for the long-lived component, indicating that rate of ethylene binding is likely to be determined by the rate of its interaction with the active center of the receptor.

1.3 Ethylene Antagonists

1.3.1 Chemical Adjuvants Counteracting Ethylene

Ethylene responses in plants can be prevented to some extent by a number of chemical adjuvants. High concentrations of sucrose, carbon dioxide, and cycloheximide delay senescence in flowers (Dilley and Carpenter 1975). Carbon dioxide is used in controlled atmosphere storage of fruits and vegetables and it has been known for many years that it is a natural inhibitor of ethylene responses. Early studies of the carbon dioxide effect suggested that it competes with ethylene in ethylene action (Burg and Burg 1967); however, direct measurement with ^{14}C-labeled ethylene did not indicate that carbon dioxide competes with ethylene for the receptor sites (Sisler 1979). Recently it was shown that carbon dioxide acts by suppressing ethylene biosynthesis (John 1997). Indoleacetic acid can prevent ethylene action under some circumstances. Application of indoleacetic or 2,4-dichlorophenoxyacetic acid to plant tissue will retard some ethylene-induced processes, but there is no evidence that they act by preventing ethylene binding, and their action also seems to be indirect (Sisler et al. 1985).

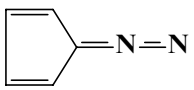
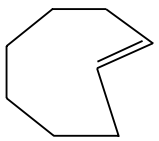
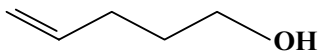
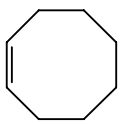
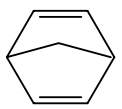
Responses to ethylene are controlled by either lowering its biosynthesis or limiting its action. A number of inhibitors of ethylene biosynthesis have been developed. Ethylene biosynthesis in plants can also be minimized by expression of a microbial ACC deaminase gene or genetic suppression of the key enzymes of the Yang cycle (Klee et al. 1991; Theologis et al. 1992). For example, ACC deaminase transgenic tomato plants that are resistant to flooding stress may be constructed by using root-specific promoters, which are also anaerobically inducible (Grichko and Glick 2001b). The practical disadvantages of genetic approaches are the necessity for development of transgenic lines of each species, which is almost impossible, and a high public concern associated with the issues of transgenic food. A non-invasive and universal way of controlling ethylene responses in plants is emerging. Indeed, plant growth can be affected in a variety of ways by plant growth-promoting bacteria expressing the ACC deaminase gene (Grichko and Glick 2001a). Because these approaches affect ethylene biosynthesis and do not protect plants from exogenous ethylene, in recent years much effort has been focused on the control of ethylene action that starts with the binding of ethylene to the receptor (Sisler 1979; Schaller and Bleecker 1995). Ag(I) ion (Beyer 1976) especially silver thiosulfate is a very effective inhibitor of ethylene action. Ag(I) ion interacts with the receptor and binds ethylene in this state (Rodriguez et al. 1999) but fails to induce response *in situ*. The Ag(I) ion is thought to occupy the binding site of the receptor (Rodriguez et al. 1999) or it might affect it in some other way. The silver ion reacts with sulfur-containing compounds and is known to deactivate enzymes by reacting with sulfhydryl groups. Silver, being a heavy metal, has been banned from use to counteract ethylene in some countries and this limits its use.

1.3.2 Ethylene Agonists That Require Continuous Exposure to Give a Response

All existing ethylene antagonists except for silver thiosulfate and cyclopropenes require continuous exposure. The fact that some alkenes inhibit ethylene responses was discovered by Sisler and Pian in 1973. 2,5-Norbornadiene (2,5-NBD) was known to form one of the most stable silver complexes. Out of curiosity, 2,5-NBD was tested on tobacco leaves, flowers, and seeds to see if it would elicit an ethylene response. It did not appear to induce an ethylene-like response but instead did seem to overcome the effect of ethylene. Several other cyclic alkenes were then tested and were effective inhibitors of ethylene action. 2,5-NBD was the best antagonist found among the cyclic alkenes. However, it required continuous exposure and had a pungent and obnoxious odor. Despite these limitations, for many years it served as an important experimental tool, and it initiated the search for ways to control the ethylene receptor. One of the more important results found with the cycloalkenes was that the level of activity appeared to depend on the

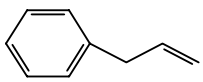
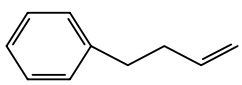
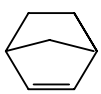
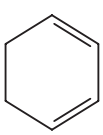
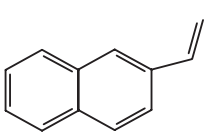
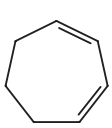
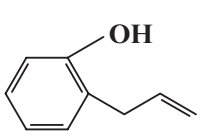
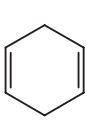
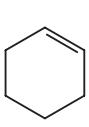
ring strain (Sisler and Yang 1984). The more strained the alkene, the better it was as an antagonist. 2,5-NBD continued to be the best alkene antagonist until *trans*-cyclooctene was discovered (Sisler et al. 1990). TCO is not much more highly strained than 2,5-norbornadiene and concentration-wise it was nearly 100 times as effective. TCO also has a very pungent and obnoxious odor and must be prepared by synthesis. It has had only limited usage. These compounds remain bound much longer than ethylene, diffusing from the binding site with a $t_{1/2}$ of 3–6 h (Sisler et al. 1990). Many ethylene responses require more than 6 h of exposure to ethylene for induction of an observable response, and the results of a single exposure would not be sufficient to be noted. This is probably the reason continuous exposure is required. Cyclic alkenes that are potent ethylene antagonists are listed in Table 1.1. Cyclopentadiene had been found to be about as effective as 2,5-NBD as an inhibitor of ethylene responses. Cyclobutene also proved to be a compound requiring continuous exposure.

Table 1.1. Inhibition of ethylene action in plants by competitive antagonists

Compound name	Structure	Plant	K_i (μL L^{-1} gas)
Diazocyclopentadiene		Carnation	0.12
trans-Cyclooctene		Banana	0.78
4-Penten-1-ol		Banana	110
cis-Cyclooctene		Banana	512
2,5-Norbornadiene		Pea Banana	170 55
Cyclopentadiene		Banana	140

(*Continued*)

Table 1.1. Inhibition of ethylene action in plants by competitive antagonists—(*cont'd*)

Compound name	Structure	Plant	K_i (μL L^{-1} gas)
Allylbenzene		Banana	189
4-Phenyl-1-butene		Banana	206
Norbornene		Pea	360
1,3-Cyclohexadiene		Pea	488
2-Vinylnaphthalene		Banana	490
1,3-Cycloheptadiene		Pea	870
2-Allylphenol		Banana	995
Cyclopentene		Pea	1,100
1,4 -Cyclohexadiene		Pea	4,650
Cyclohexene		Pea	6,060