Effect of Ethylene on Root Architecture in Peas

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Abstract

Root architecture describes root growth over time and space. Prior studies have examined the effects of ethylene and nutrient deficiency using ethylene precursors or inhibitors in combination with nutrient deficiency. To test if ethylene gas alone could alter root architecture in young pea plants, a 30 ppb flux of ethylene was maintained through a column root zone. Although not statistically significant, the data trend shows that roots grown without ethylene were longer, had more lateral branches, and supported larger shoots. This is contrary to literature that shows ethylene induces root growth under nutrient deficiency.

Introduction

Soil resources such as water, oxygen and mineral nutrients are not uniformly distributed. Plants have evolved a wide variety of root systems matched to the vast array of soil environments. Root system architecture (RSA), the spatial configuration of root systems, describes the positioning of meristematic, lateral and hair growth in a root system over time and space. Thus, the ability of a root system to efficiently explore and exploit resources in different soil conditions is described by RSA. Thus, the primary measures of RSA root topology and distribution. RSA varies considerably both across and within species and is heavily influenced by the soil environment. The RSA of a plant is a trade-off between carbon invested in roots and resources returned. Therefore,

changes in RSA necessitated by soil resource limitations negatively impact plant growth and crop yields.

Ethylene plays an active role in determining the response of a root system to external nutrient levels and thus a key role in architecture (Ho *et al.*, 2005). Ethylene-RSA interaction research has focused on RSA response while limiting an essential nutrient (review: López-Bucio *et al.*, 2003). Phosphorous, iron and sulfur deficiencies (since they are all immobile or difficult elements to obtain from the soil solution) have attracted the most attention (López-Bucio *et al.*, 2003).

Phosphate-ethylene-RSA interactions have been studied in *Zea mays* (He, *et al.*, 1991), *Phaseolus vulgaris* L. (Borch *et al.*, 1999), and *Arabidopsis thaliana* (Zhang *et al.*, 2003; Ma *et al.*, 2001). Based on earlier work with hypoxia and flooding, He *et al.* (1991) sought indications that ethylene may play a role in *Zea mays* RSA. They found that N or P deficient roots were more sensitive to exogenous ethylene precursors than those that were not.

Subsequent research conducted using common bean (*Phaseolus vulgaris* L.) showed that when endogenous ethylene production was reduced by aminoethoxyvinylglycine (AVG), there was a significant interaction between P availability and ethylene treatment, indicating that P deficiency changes tissue responsiveness to ethylene. Thus, ethylene could control RSA by either changing synthesis or changing tissue response (Borch *et al.*, 1999).

Under low P conditions *Arabidopsis* roots had 5x more root hairs than roots grown in high P concentrations. The application of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) or the ethylene synthesis inhibitor

amino-oxyacetic acid (AOA) increased root hair density under high P conditions. Root hair density was not affected by the application of either ACC or AOA in the low P condition (Ma, *et al.*, 2001). Since AOA failed to decrease root hair density in either condition, however, the authors suggest that ethylene must be using a different mechanism in the high P condition to trigger hair growth and that ethylene is not important for the conveyance of the “low P” signal (Ma, *et al.*, 2001). However, subsequent work using the ethylene synthesis inhibitor AVG and ethylene insensitive mutants suggested a more complex interaction exists than previously thought (Zhang *et al.*, 2003). Indeed, Zhong *et al.*, (2003) conclude that P deficiency alters the sensitivity of the signaling pathway linking ethylene to cell growth and division.

Iron-ethylene-RSA interaction studies have had history similar to phosphate-ethylene-RSA studies. Young cucumber plants (*Ccumis stivus* L. cv. Ashley) grown in the presence of ethylene synthesis and action inhibitors had decreased Fe deficiency responses, ferric-reducing capacity and subapical root swelling (Romera and Alcåntara 1994). Iron deficiency and ethylene were also able to stimulate root hair growth in *Arabidopsis* similar to that seen with P deficiency (Schmidt, *et al.*, 2000). Proteoid root formation in Fe deficient conditions was inhibited when AVG and other ethylene synthesis or perception inhibitors were applied to growth chamber grown *Casuarina glauca* seedlings (Zaid, *et al.*, 2003).

The papers reviewed above provide a sample of current and past investigations into the role ethylene plays in determining root architecture. It is

apparent that root architecture changes in response to: 1) nutrient deficiencies and 2) exogenously applied ethylene precursors. All the papers used chemical inhibitors of ethylene synthesis or action to demonstrate changes in RSA. Also, no researchers have compared the effect of ethylene gas on root architecture without a nutrient deficiency. Using a root column similar to that described in Borch *et al.* (1999) I tested the following hypothesis:

- Exogenous ethylene at 30 ppb will alter RSA in young pea plants.

Materials and Methods

Earligreen pea seeds were imbibed overnight in germination boxes. Fully swelled seeds were transferred to 30 cm long columns (Figure 1). Three replicate columns were planted at 0 ppb (control) and three at 30 ppb (treatment) levels of ethylene. Columns were watered with a complete nutrient solution (Peter's Hydro-Sol 1:200 dilution) thrice daily. An air flux of 50 mL min^{-1} was maintained in each column. Columns were sealed and ethylene treatments were started two days post emergence (DPE) and continued until harvest. Ethylene levels in a central reservoir and the columns were monitored using a gas chromatograph equipped with a flame ionization detector and Porapak Q column. Ethylene treated air was distributed from the central reservoir to the columns. Plants were harvested 10 DPE. The length of the radicle and the number of lateral roots were recorded.

Results

Ethylene applied at 30 ppb to the root zone did not significantly affect primary root length, the number of lateral roots or root fresh mass (Table 1). Shoot length was not affected (Table 1, Figure 2). Root hairs were not present in either treatment (Figure 3). Tertiary roots were observed in the control group and not the treatment group, but this was not quantified (Figure 3).

Discussion

Several problems plagued this experiment. First, it was not always clear that the correct amount of ethylene was distributed to each column. Although the distribution reservoir was constant, the column levels occasionally fluctuated by 5-10 ppb. Second, one seed failed to germinate in the control columns, thus skewing the statistical analysis.

Although the results were not significant the trend indicates that plants grown without ethylene had higher fresh mass, radicle length, lateral root number and shoot length. The observation that tertiary roots were present in the control and not treated roots supports the measurements. This is contrary to data gleaned using precursors and nutrient deficiency. This suggests that under nutrient sufficient conditions ethylene may act as a root growth inhibitor. This would prevent the plant from investing carbon in unneeded root growth.

Future work should include ethylene at multiple higher levels in order to validate and further quantify these observations. The introduction of ACC positive controls and combinations with selected nutrient deficiency would further define the role ethylene plays in root growth regulation.

Literature Cited

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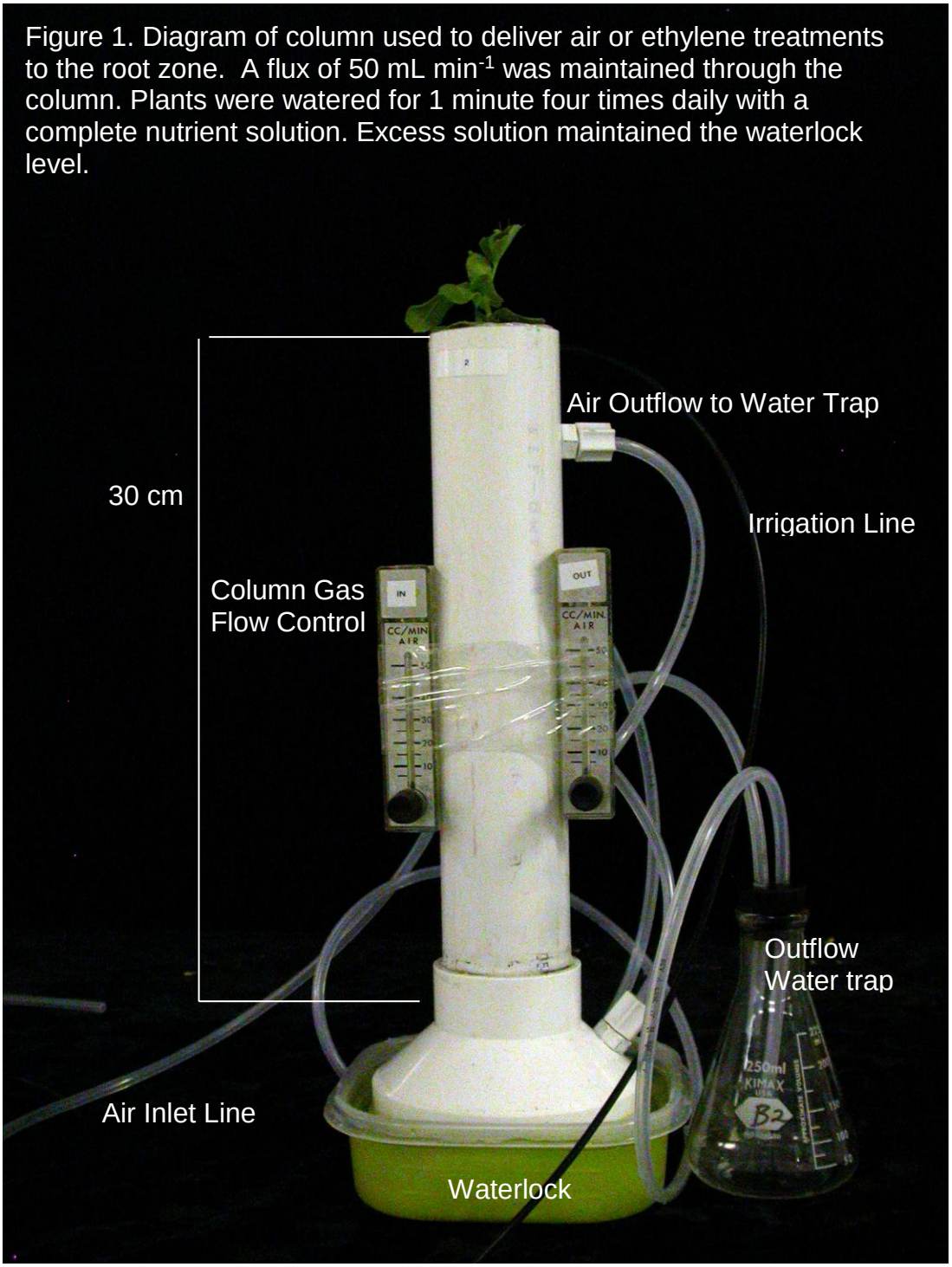

Figure 1. Diagram of column used to deliver air or ethylene treatments to the root zone. A flux of 50 mL min^{-1} was maintained through the column. Plants were watered for 1 minute four times daily with a complete nutrient solution. Excess solution maintained the waterlock level.

Table 1. Effect of 30 ppb ethylene on root growth of 10 DPE Earligreen pea plants. Significantly different measurements are bolded.

Parameter	**0 ppb mean**	**30 ppb mean**	**p-value** (ANOVA, α=0.05)
Root Fresh Mass (g)	3.8	3.3	0.588
Radicle Length (cm)	31.5	29.7	0.334
Number of Lateral Roots	85.0	80.0	0.705
Shoot Length	7.5	**7.0**	**0.272**

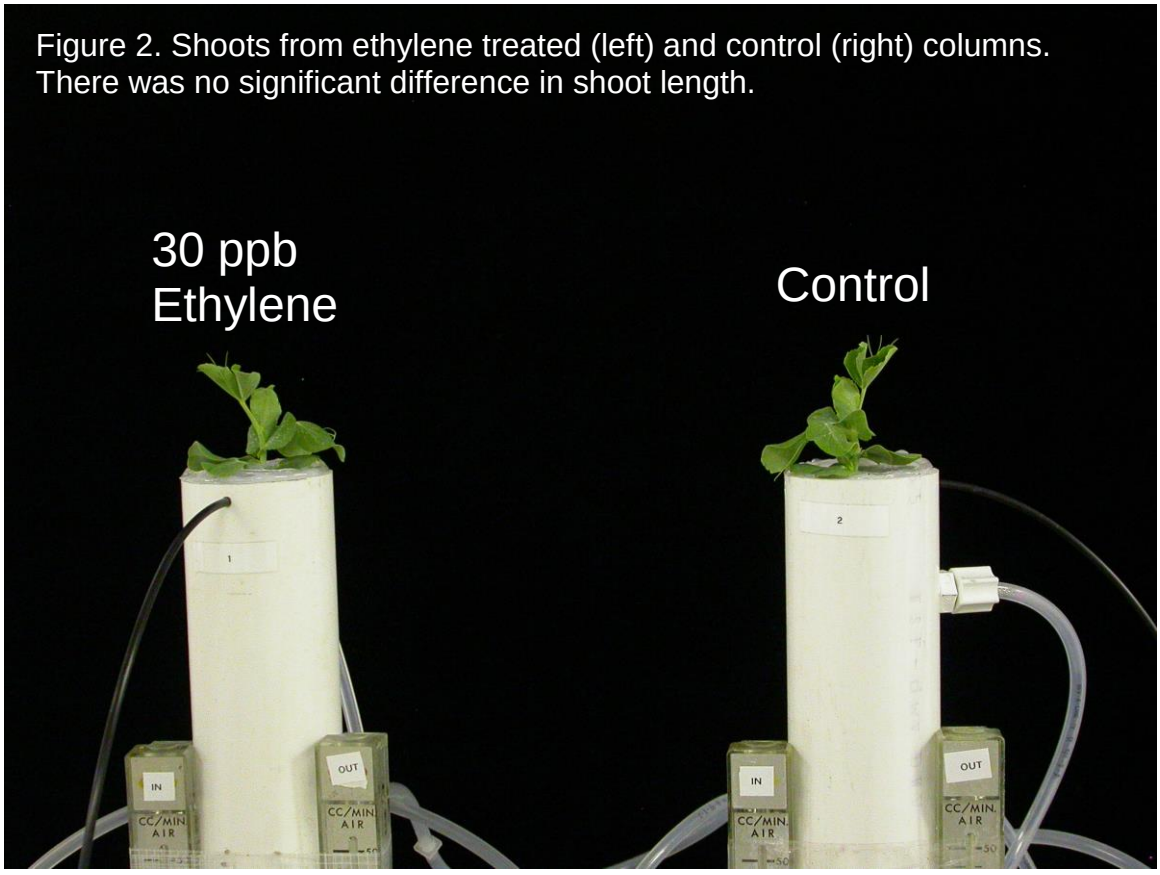

Figure 2. Shoots from ethylene treated (left) and control (right) columns. There was no significant difference in shoot length.

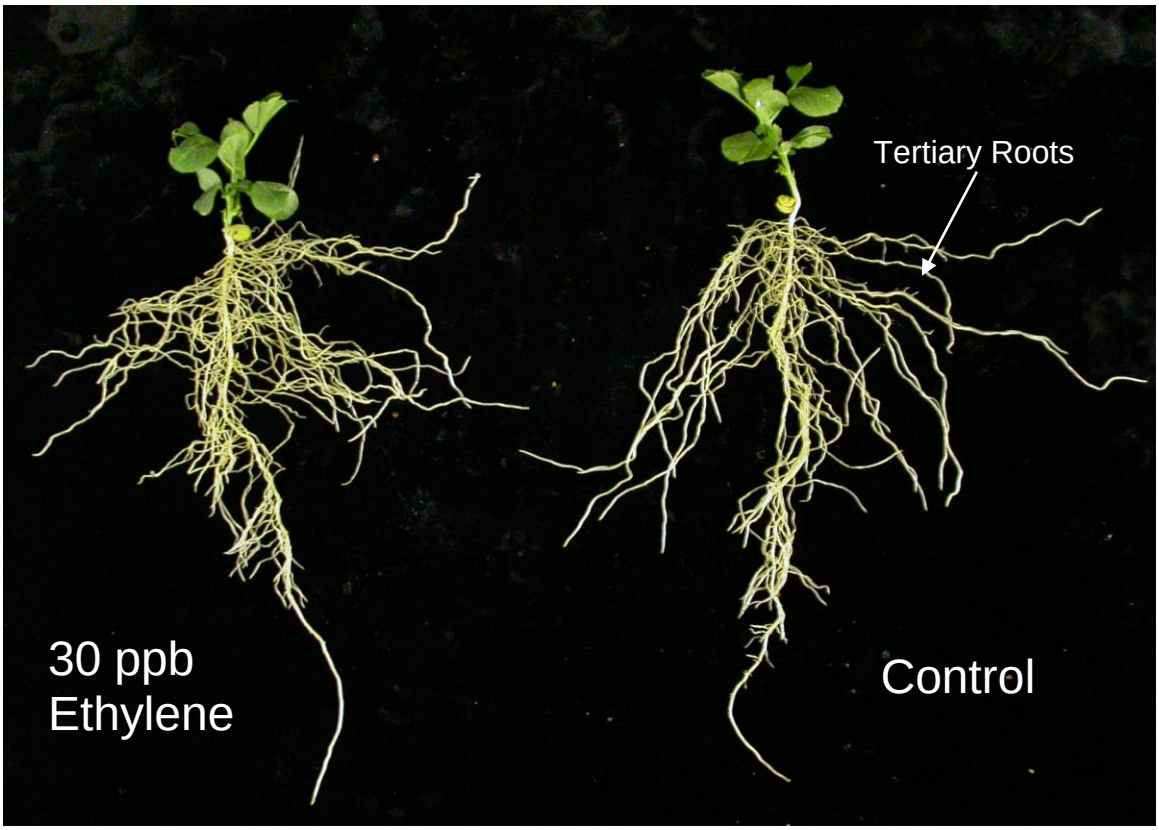

Figure 3. Roots and shoots from ethylene treated (left) and control (right) columns. Although the control treated roots had higher average root mass, root length and lateral root number the difference was not significant. Control roots had tertiary root tissue whereas treated roots did not. This effect was not