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Morphological alterations of pea (*Pisum sativum* cv. Sparkle) arbuscular mycorrhizas as a result of exogenous ethylene treatment

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Abstract Little is known about the role of phytohormones in the formation of arbuscular mycorrhizas (AM). Although the involvement of ethylene in AM formation is unclear, it is considered very important for several aspects of plant growth and development. The effect of a suspected inhibitory level of ethylene was investigated to help elucidate its role in regulating the formation of AM. In particular, the morphology of AM fungal structures at various stages of the colonization process was documented. Exogenous application of 5.5 ppm ethylene to the substrate resulted in typical morphological changes to *Pisum sativum* and a significant reduction in the colonization of roots by the AM fungus *Glomus aggregatum*. Elevated substrate-ethylene did not affect the number of appressoria formed; however, it did result in the formation of abnormal appressoria, which appeared swollen and highly branched. Deformation of appressoria was correlated with a reduction of AM fungal entry into the root tissue, resulting in less colonization by intraradical hyphae and arbuscules. Colonization generally proceeded normally provided the fungal hyphae breached the epidermis, although the extension of colonization units was restricted.

Keywords Appressoria · Colonization · Confocal microscopy · *Glomus aggregatum* · Plant hormones

Introduction

Recently, the role of phytohormones in the formation of arbuscular mycorrhizas (AM) has attracted attention. A potential role for hormones of host-plant origin in the regulation of AM formation is recognized (Beyrle 1995; Hirsch et al. 1997; Lynch and Brown 1997; Ludwig-Müller 2000) but a definitive role for the effects of any of these substances is lacking (Beyrle 1995; Ludwig-Müller 2000). Evidence that ethylene, a gaseous phytohormone with various roles in plant growth and development (Jackson 1991; Abeles et al. 1992), could play a role in AM formation does exist (reviewed by Ludwig-Müller 2000).

Investigations of the effects of ethylene on AM fungi alone are limited. Ethrel, an ethylene-releasing compound (Azcon-Aguilar et al. 1981), and ethylene gas (Ishii et al. 1996) inhibit spore germination and hyphal growth from spores. However, at very low levels of ethylene (0.01–0.1 ppm), Ishii et al. (1996) report stimulation of both spore germination and resulting hyphal growth. This stimulation of hyphal growth was retarded in the presence of ethylene absorbents (Ishii et al. 1996).

Several studies have addressed the effects of ethylene on the AM symbiosis. Experiments with ethrel indicate that ethylene plays an inhibitory role in AM formation, i.e., treatment of host plants with this compound depresses AM fungal colonization (Azcon-Aguilar et al. 1981; Morandi 1989). However, the actual concentration of ethylene applied was not determined in either study. Ishii et al. (1996) reported inhibition of AM formation as a result of applying 1 ppm exogenous ethylene gas in 2 l of air once a day; however, at 0.05 ppm ethylene, significant stimulation of AM formation occurred. Thus, it seems ethylene can either inhibit or promote the formation of AM depending on its concentration. Indirect evidence for the involvement of ethylene in AM formation also exists. For example, El Ghachtouli et al. (1995) suggest that increased mycorrhizal infection frequency in peas treated with polyamines could be a function of inhibitory effects of these compounds on ethylene production. Similarly, Nadian et al. (1998) speculated that the depressed AM fungal colonization of clover grown in compacted soil may be due to increased production of ethylene by impeded roots.

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The objective of this study was to describe the morphology of AM formed in a substrate permeated with a suspected inhibitory level of exogenous ethylene, as previous studies have only reported quantitative differences in colonization. To accomplish this, we applied a continuous flow of 5.5 ppm ethylene gas to the pea (*Pisum sativum* L. cv. Sparkle) root-*Glomus aggregatum* Schenck and Smith emend. Koske system and characterized its effects on colonization level and the morphology of AM fungal structures formed. Although 5.5 ppm ethylene may seem quite high, we wanted to ensure that we applied an inhibitory concentration. Whereas soil-ethylene levels in nature are generally ≤0.2 ppm, waterlogging, soil compaction, and organic materials can all cause ethylene levels to increase up to 10 ppm (Abeles et al. 1992).

Materials and methods

Cultivation of mycorrhizal plants

Seeds of *P. sativum* cv. Sparkle were surface-sterilized in an aqueous solution of 8% household bleach (5.25% sodium hypochlorite) for 5 min followed by three rinses (1 min each) in sterile water. They were then placed in a fresh rinse of sterile water and left to imbibe overnight in the dark. Imbibed seeds were transferred to sterile Petri plates containing filter paper moistened with water; plated seeds were kept in the dark for 3 days to germinate. Homogeneous 3-day-old seedlings were selected and planted one per Conetainer (560 ml volume; Ray Leach Conetainer Nursery, Canby, Oregon) in inoculated substrate. Each Conetainer had eight holes (5 mm diameter) drilled midway along its length to facilitate exposure of the substrate to ethylene. The inoculated substrate consisted of autoclaved (120°C for 20 min) Turface (calcined montmorillonite clay; Applied Industrial Materials, Buffalo Grove, Ill.) and peat (Greenworld Garden Products, Pointe Sapin, New Brunswick) (3:1, v/v) mixed with pot-cultured inoculum of the AM fungus, 13:1 Turface/peat:inoculum (v/v). The pot-cultured inoculum was composed of desiccated leek roots colonized by *G. aggregatum* in a Turface carrier. Plants were cultivated in a growth-room with a day/night cycle of 22°C/18°C for 16 h/8 h. Control and ethylene-treatment plants were exposed to mean light intensities of 346 and 369 µmol m^{-2} s^{-1} photosynthetic photon flux density respectively. The inoculated substrate in each Conetainer was saturated with sterile water 1 day prior to planting; each plant was given 30 ml sterile water every 2 days. Sterile low-phosphate nutrient solution (30 ml) was substituted for water once per week. The low-phosphate nutrient solution (pH 6.8) contained 54.4 mg l^{-1} KH_2PO_4, 590.4 mg l^{-1} $Ca(NO_3)_2.4H_2O$, 349.0 mg l^{-1} K_2SO_4, 246.5 mg l^{-1} $MgSO_4.7H_2O$, 82.0 mg l^{-1} Fe(III) EDTA, 3.73 mg l^{-1} KCl, 1.55 mg l^{-1} H_3BO_3, 0.58 mg l^{-1} $ZnSO_4.7H_2O$, 0.34 mg l^{-1} $MnSO_4.H_2O$, 0.13 mg l^{-1} $CuSO_4.5H_2O$, 0.12 mg l^{-1} $Na_2MoO_4.2H_2O$. Plants were cultivated for 25 days before root systems were collected.

Application of exogenous ethylene

Exogenous ethylene was applied using the apparatus described by Lee and LaRue (1992a, 1992b) with slight modifications. Prepared Conetainers were racked into a 125 l opaque-plastic chamber by fitting them into a wood frame that was sealed to the open end of the chamber using a rubber gasket and duct tape. A continuous flow of ethylene gas in air was added to one chamber (treatment chamber) whereas a constant flow of air only was added to another (control chamber). In each case, the gas and/or air were released into the chamber in a shallow depth of sterile water for even distribution; however, all shoots remained in the same open atmosphere of the growth-room. Ethylene gas was introduced into the treatment chamber by merging an ethylene gas line with an air line, yielding a mean substrate-ethylene concentration of 5.5 (±0.49 SE) ppm for the duration of the experiment. The amount of ethylene gas merged with the air line was adjusted using a microvalve (Nupro, Willoughby, Ohio). The air line was established by using a pump to collect air from the growth-room and then passing it through a column containing potassium permanganate adsorbed to clay (an ethylene absorbent from Purafil, Atlanta, Ga.). This ethylene-scrubbed air was also used to provide airflow into the control chamber. The flow rate of air through each line (one per chamber) was adjusted to 2 l min^{-1} using flow meters (7631T-603; Matheson gas products, Whitby, Ontario).

The level of substrate-ethylene within the Conetainers in both chambers was determined and monitored throughout the course of the experiment. A 14 cm length of 0.635 cm internal diameter PVC tubing, capped with a septum, was inserted into one Conetainer from each chamber during preparation. Syringe air samples (1 ml each) were collected regularly during the experiment and injected into a Perkin-Elmer 3920B gas chromatograph (GC) equipped with a flame ionization detector. The GC was fitted with a 1.83 m stainless steel column (0.3175 cm outer diameter) packed with Porapak N (80/100 mesh). Samples were analyzed at a column temperature of 115°C and a carrier gas (N_2) flow rate of 42 psi. Air samples from the growth-room atmosphere (entire volume of air in the room exchanged for outdoor air 6 times h^{-1}) were also collected and analyzed to ensure that the air surrounding plant shoots was free of ethylene.

Root collection and processing

Before root systems were collected, various growth parameters were measured on control and ethylene-treated plants. Root-system measurements consisted of length of the primary root, length of the longest secondary root, number of secondary roots in the upper 3 cm of the primary root, and fresh weight. Measurements of the shoot included height, length of the third internode, number of nodes, and fresh and dry weights (shoots were dried in an oven at 50°C for 7 days). Data presented are mean values from six plants (unless indicated otherwise) ±SE. Statistical differences between ethylene-treated and control plants were determined by applying Student's *t*-tests or Mann-Whitney rank sum tests (when data failed the equal variance test).

Root systems were excised just below the cotyledons and processed according to Brundrett et al. (1994) with some modifications. They were fixed in 50% ethanol for a minimum of 24 h, after which all of the secondary roots were excised from the primary root and chopped into fragments approximately 1 cm in length. A sub-sample of approximately 60 fragments was then collected randomly from each root system. All sub-samples were cleared by autoclaving in 5% KOH (w/v) and rinsed in water before being placed in 0.1 N HCl for 1 h. After acidification, the root fragments were stained for 3 days at room temperature in the dark in a solution consisting of equal parts of 1% aqueous acid fuchsin (w/v), 85% lactic acid, and glycerol. After staining, root fragments were placed in 100% glycerol for de-staining (minimum 24 h). In an attempt to optimize staining for laser scanning confocal microscopy, additional sub-samples were taken from control root systems and stained with 0.01% acid fuchsin (Kormanik and McGraw 1984). Processing was carried out as described above except that the acidification step consisted of a 5 min rinse in 1% HCl (v/v) and root fragments were stained for 1 h in an oven at 55°C.

Quantification of AM fungal colonization

From each root system sub-sample, 45 de-stained fragments were mounted (approximately 15 fragments per slide) in glycerol on glass slides and covered with 20×50 mm (no. 1) coverslips. Prepared slides were scanned at ×312 total magnification using a Leitz Wetzlar compound light microscope equipped with Nomar-

ski optics (Plan ×25, NA 0.50) and a ×10 ocular with a crosshair. Each intersection between a root and the ocular crosshair was scored for the presence of at least one appressorium, intraradical hypha, and/or arbuscule using the magnified intersections method of McGonigle et al. (1990). Values for the fungal structures scored are expressed as a percentage of the total intersections scored (approximately 150 per root system).

Because of the very low abundance of appressoria observed, slides were re-scored for appressoria only. The magnified intersections method was modified in an attempt to gain further insight into the development of fungal hyphae from appressoria. The same procedure was followed except that during scanning of the slides, the entire field of view (rather than just root-crosshair intersections) was checked for appressoria; the field of view at ×312 magnification encompassed 800 µm of a root-fragment length. Upon observation, appressoria were scored and hyphal development was categorized as being restricted to the root surface, entering but not exiting the epidermal layer, or progressing into the cortical tissue of the root. Values for hyphal development from appressoria are expressed as a percentage of the total number of appressoria scored. It was anticipated that this modified quantification method would enable a determination of whether or not 5.5 ppm exogenous ethylene in the substrate affected AM fungal colonization at a specific stage of development.

Each colonization or hyphal development value represents the mean of eight plants ±SE from one representative experiment. To determine significant differences between ethylene-treated and control plants, Student's *t*-tests were applied unless data failed the normality test, in which case a Mann-Whitney rank sum test was applied. All statistics were performed using SigmaStat 2.03 software (SPSS, Chicago, Ill.).

Qualitative description of AM fungal colonization

Slides prepared for quantification of AM fungal colonization were subsequently used for laser scanning confocal microscopy. Coverslips were sealed with nail polish before specimens were viewed with a BioRad MRC-600 laser scanning confocal microscope. This microscope was equipped with a krypton-argon mixed gas laser interfaced with Nikon fluorescence optics. AM fungal colonization was viewed using a ×60 oil immersion objective (Plan Apo, NA 1.4). A 568 nm excitation wavelength was used to irradiate the samples and images were collected using the photomultiplier tube 1 detector for emission wavelengths >560 nm. Several series of optical sections were collected at 1.0 µm intervals and various numbers of these sections were subsequently used to reconstruct *z*-series composite images. Light micrographs were taken on Ilford PANF 50 ASA film using a Reichert Microstar IV compound light microscope (×40/ NA 0.66 and ×10/ NA 0.25 Plan Achro objectives). The plate was prepared using Adobe Photoshop 5.0 software (Adobe Systems, Seattle, Wash.).

Results and discussion

Ethylene effects on plant growth

In this study, the response of pea plants to 5.5 ppm exogenous substrate-ethylene included morphological changes (Table 1) consistent with previous reports on ethylene effects (Abeles et al. 1992; Lee and LaRue 1992a) and typical of the "triple response". This phenotype is characterized by decreased elongation (extension), increased radial expansion, and horizontal growth (Abeles et al. 1992). Roots and shoots were significantly shorter than those of control plants; the reduction in shoot height was a function of shorter internodes, not fewer nodes (Table 1). Also, the roots and shoots weighed significantly less than those of control plants (Table 1). Significantly fewer secondary roots were initiated in the top 3 cm of primary roots (Table 1) and root systems were highly contorted with soil adhering to them very tightly.

As reviewed by Shibaoka (1994), in pea stems, ethylene alters the orientation of microtubules, a change that causes new cellulose microfibrils to be deposited in a longitudinal orientation. It is thought that this reorientation is the cause of increased radial expansion and decreased longitudinal extension (Shibaoka 1994). The triple response is not limited to shoots as all three of these growth responses, in addition to others, are also known to occur in roots treated with exogenous ethylene (see Feldman 1984; Jackson 1991). One ethylene-mediated mechanism that may be involved in the regulation of AM formation could be the alteration of host and/or AM fungal cytoskeletal components. While ethylene effects on host microtubule and cellulose microfibril orientation are known, nothing is known about alterations to the cytoskeleton of AM fungi. Now that immunolabelling of AM fungal cytoskeletal components has been demonstrated (Åström et al. 1994; Timonen et al. 2001), it will be possible to investigate whether ethylene alters fungal cytoskeletal components.

Table 1 Growth parameters measured (25 days after planting/inoculation) for *Pisum sativum* cv. Sparkle plants inoculated with *Glomus aggregatum* and cultivated in the absence or presence of 5.5 ppm exogenous substrate-ethylene. Each value represents the mean of six plants ±SE. Each parameter was compared between treatments using Student's *t*-test unless data failed the equal variance test, in which case a Mann-Whitney rank sum test (*MW*) was applied

Growth parameter	0 ppm Ethylene	5.5 ppm Ethylene	*P*-value
Length of primary root (mm)	273.8±8.18[a]	78.7±6.31	<0.001
Longest secondary root (mm)	240.8±10.44	51.3±3.84	<0.001
No. of secondary roots in top 3 cm of primary	13.7±1.20	10.0±0.78	0.028
Root fresh weight (g)	4.83±0.30	2.45±0.28	<0.001
Shoot height (mm)	182.3±6.05	133.3±13.52	0.002 (MW)
Length of third internode (mm)	16.7±1.63	10.4±1.55	0.019
Number of nodes	12.0±0.00	10.5±0.49	0.065 (MW)
Shoot fresh weight (g)	3.53±0.11	1.72±0.24	<0.001
Shoot dry weight (g)	0.61±0.01	0.27±0.05	<0.001

[a] 5 plants only

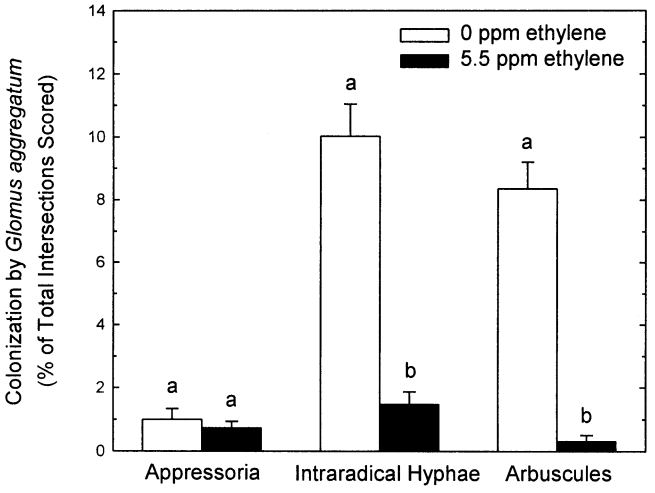


Fig. 1 Colonization of pea (*Pisum sativum* cv. Sparkle) roots by *Glomus aggregatum* 25 days after inoculation as determined by the magnified intersections method. Significant differences in the mean prevalence of the noted fungal structures are indicated *by different letters* between pairs of bars ($P<0.001$, Student's *t*-test, $n=8$). *Error bars* represent SE

AM fungal colonization

Although the application of 5.5 ppm exogenous ethylene to the substrate had no significant effect on the prevalence of appressoria formed on the surface of roots (Fig. 1, Table 2), there was a significant reduction in the amounts of both intraradical hyphae and arbuscules (Fig. 1). This finding supports earlier reports of ethylene-induced inhibition of root colonization by AM fungi (Azcon-Aguilar et al. 1981; Morandi 1989; Ishii et al. 1996). However, the design of our experiment does not enable separation of direct effects of ethylene on *G. aggregatum* from indirect ethylene effects on AM colonization via direct effects on pea morphology and anatomy.

The ethylene treatment resulted in morphological changes at various stages of AM development in our system. The most striking of these alterations was the deformation of *G. aggregatum* appressoria. In contrast to the characteristic elliptical appressoria (Fig. 2) formed on the surface of control-treatment roots, those formed on ethylene-treated roots were often highly deformed (Fig. 3 and Table 2). Deformed appressoria exhibited extensive branching, swelling, and septa formation; they appeared enlarged compared to typical appressoria. These deformed appressoria are reminiscent of those that form on myc$^-$ *Medicago* mutants (illustrated in Bradbury et al. 1991, 1993) and the rmc mutant of *Lycopersicon esculentum* (illustrated in Barker et al. 1998). Each of

Figs. 2–9 Extended focus laser scanning confocal (Figs. 2, 3, 5, 6, 7) and light (Figs. 4, 8, 9) micrographs of colonization events between *G. aggregatum* and roots of pea (*P. sativum* cv. Sparkle). Colonization occurred either in the absence (Figs. 2, 6, 8) or presence (Fig. 3, 4, 5, 7, 9) of 5.5 ppm exogenous substrate-ethylene. Scale bars=25 µm (Figs. 2, 3, 4, 5, 6, 7) and 100 µm (Figs. 8, 9) ▶

Fig. 2 Characteristic elliptical appressoria. Each appressorium formed at the terminus of an extraradical hypha (*eh*) and commonly between two adjacent files of epidermal cells (*arrowhead*)

Fig. 3 A deformed appressorium exhibiting profuse branching and appearing swollen and septate (*arrow*)

Fig. 4 Swollen hyphae (*arrowheads*) constricting as they passed from the epidermal (*ep*) to the underlying cell layer and expanded once again

Fig. 5 Extensively branched hypha (*) in the inner cortical tissue. Note constriction (*arrowheads*) of the hyphae as they pass from one cell to another

Fig. 6 An arbuscule of typical *Arum*-type morphology; a thick trunk hypha (*arrowhead*) gave rise to several progressively finer branches (*arrow*)

Fig. 7 Treatment with ethylene altered cortical cell shape from cylindrical to cubical (compare with Fig. 6). This alteration in host cell morphology resulted in the apparent compression of arbuscules; however, arbuscules seemed normal in that they were composed of trunk hyphae (*arrowheads*) with numerous fine branches (*arrow*)

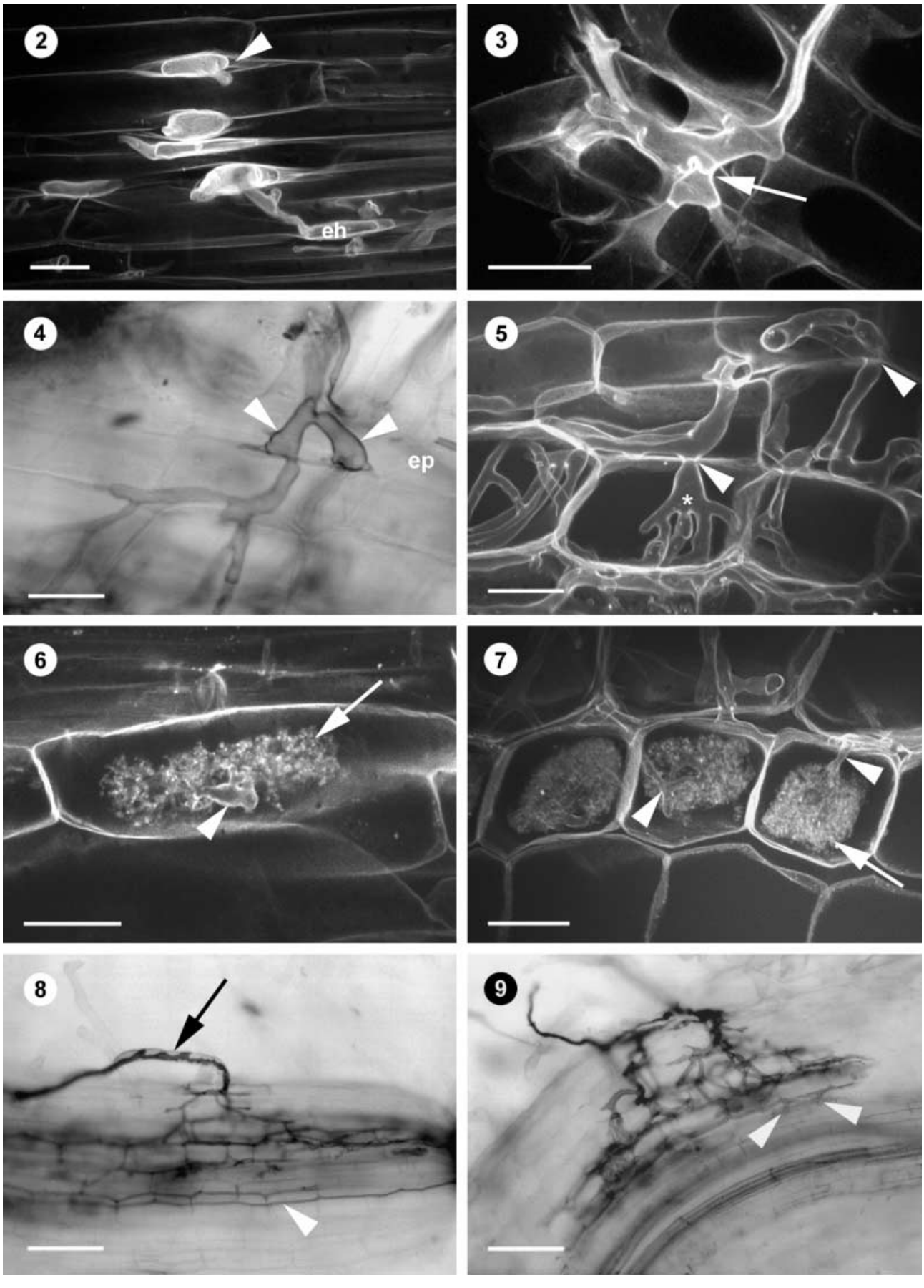
Fig. 8 A characteristic colonization unit with fungal hyphae extending along a large portion of the longitudinal axis of the root. This large expanse of fungus within the root tissue resulted from its entry into the intercellular spaces (*arrowhead*) where there is little impedance to fungal growth. Occasionally, roots were colonized via a root hair (*arrow*) rather than by the formation of an appressorium between non-hair epidermal cells

Fig. 9 A colonization unit in an ethylene-treated root. Colonization units were consistently restricted in terms of the spread of the fungus along the long axis of the root. Progression of the fungus towards the inner cortex of the root, where arbuscules (*arrowheads*) formed without difficulty, did not seem to be impeded as a result of the ethylene treatment

Table 2 Prevalence of morphology and hyphal development from *G. aggregatum* appressoria formed on pea (*P. sativum* cv. Sparkle) roots determined using a modification of the magnified intersections method. Each value represents the mean of eight plants ±SE. Comparisons between ethylene treatments were made using Student's *t*-tests unless data failed the normality test, in which case a MW rank sum test was applied. *FOV* Fields of view

	0 ppm Ethylene	5.5 ppm Ethylene	*P*-value
Prevalence of appressoria			
% Of total FOV with appressoria	3.67±0.64	4.67±0.88	0.372
Morphology of appressoria			
%[a] With branched and swollen morphology	3.57±3.57	61.25±8.84	<0.001 (MW)
Hyphal development from appressoria			
%[a] Restricted to root surface	17.25±6.30	59.59±9.99	0.003
%[a] Blocked in epidermis	4.86±3.23	4.59±3.02	0.959 (MW)
%[a] Reaching inner cortex	77.88±7.84	35.84±10.44	0.006

[a] Of total appressoria observed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these mutants exhibits a root surface block to AM fungal colonization as do pea plants treated with exogenous ethylene (this study). In the *P. sativum* mutant E107, colonization is blocked both at the root surface and within the epidermal cell layer, where hyphal swelling occurs (Resendes et al. 2001). Similarly, AM fungal hyphae become swollen and deformed upon reaching the epidermal cell layer in the *LjSym4* mutants of *Lotus japonicus* (Bonfante et al. 2000). In the present study, treatment with exogenous ethylene resulted in altered fungal morphology both at the root surface and within the outermost layers of root tissue and resulted in decreased colonization. The similarities between AM mutant morphology and that of AM developed in the presence of exogenous ethylene suggest that ethylene may play a significant role in these host mutations.

Interestingly, the development of hyphae from appressoria formed on ethylene-treated roots was affected by the ethylene treatment; a significantly higher percentage of appressoria was blocked at the root surface, i.e., no hyphae developed from 60% of the appressoria (Table 2). Correlation of this block with the deformation of appressoria indicates that ethylene may alter the composition of the outer periclinal walls of epidermal cells and/or affect fungal development directly, hindering entry by the fungus. The root surface block resulted in significantly fewer hyphae reaching the inner cortex of the root (Table 2), limiting root colonization by intraradical hyphae and arbuscules. No significant difference was observed in the percentage of appressoria giving rise to hyphae blocked in the epidermal cell layer (Table 2).

When *G. aggregatum* hyphae were able to breach the surface of ethylene-treated roots, their morphology inside the root tissue was also quite different from that of hyphae observed in control roots. The intraradical hyphae present in control roots exhibited typical *Arum*-type morphology (Smith and Read 1997) as they progressed from the root surface towards the inner cortex. In particular, the hyphae remained of relatively uniform diameter as they colonized the root tissue, although they did appear to constrict slightly as they passed from one cell layer to another. In contrast, hyphae inside the epidermal or outermost cortical cell layers of ethylene-treated roots often became swollen before constricting to pass from one cell layer to another (Fig. 4) indicating that hyphae likely encountered resistance in this outermost region of the root as well as at the root surface. Whereas non-extensive branching of fungal hyphae within host cells was observed in control-treatment roots, hyphae within cortical cells of ethylene-treated roots were often highly branched (Fig. 5).

Arbuscules formed within cortical cells of control-treatment roots were initiated from thick trunk hyphae that branched dichotomously into finer branches (Fig. 6). Arbuscules formed within cortical cells of ethylene-treated roots exhibited similar morphology with the exception that they were rather compressed (Fig. 7). This compressed appearance is likely a result of ethylene-induced host-cell shape alteration; three-dimensional measurements of arbuscules (Dickson and Kolesik 1999) may provide a definitive answer as to whether they actually exhibit altered morphology. Single cortical cells containing two arbuscules were observed occasionally in both control and ethylene-treated roots.

Colonization units, typical of *Arum*-type AM, extended considerable distances along the longitudinal axis of control-treatment roots (Fig. 8). Hyphae commonly entered the intercellular spaces of the cortex, allowing rapid, unimpeded colonization to take place. In contrast, colonization units in ethylene-treated roots were limited in their longitudinal expanse (Fig. 9). However, the progression of fungal hyphae towards the inner cortex of the root seemed to occur without difficulty. Hyphae were observed within intercellular spaces of ethylene-treated roots but their growth appeared to be restricted in this microhabitat. The lack of longitudinal extension of colonization units may have resulted either from the extensive branching exhibited by intraradical hyphae and/or the presence of an intercellular environment unfavourable to fungal growth.

In conclusion, 5.5 ppm exogenous ethylene applied to the substrate elicits profound changes to the morphology of pea plants. As a result of this ethylene treatment, two critical steps in the colonization of pea roots by the AM fungus *G. aggregatum* were limited. First, a block at the root surface was evident and correlated with the deformation of fungal appressoria. Second, intraradical extension of the AM fungus along the longitudinal axes of roots was restricted. Treatment of host plants with exogenous substrate-ethylene did not affect the number of appressoria formed on root surfaces but it did hinder the progression of the fungus into the inner cortex of the root. It is likely that other phytohormones, either alone or in concert with ethylene, play critical roles in AM formation. Indeed, more work is needed in detailing the mechanisms by which ethylene influences AM formation.

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