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Ethylene-induced root coiling in tomato seedlings

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Abstract. Roots of tomato seedlings can be induced to coil by treatment with ethylene. The extent of coiling is dependent on the level of ethylene to which the seedlings are exposed and can be prevented by the incorporation of Ag ions into the growing medium. In contrast to all other tomato mutants examined, roots of the mutant diageotropica do not reorientate their growth in response to ethylene. The results of an agar penetration test indicate that roots of this mutant are agravitropic. The relationship between gravitropism and root coiling, and the origin of the ethylene modified growth pattern is discussed.

Introduction

An effect of ethylene on root growth has been documented by a number of workers. The extent of the response varies depending on both the species examined and the concentration of ethylene to which the tissue is exposed. For instance, Smith and Robertson [14] reported that root extension by some cultivars of rice could be stimulated by $0.1\,\mu l\,l^{-1}$ of ethylene, while root growth of tomato and barley was inhibited at this concentration. At levels lower than this ($< 0.02\,\mu l\,l^{-1}$) root elongation of intact tomato seedlings was promoted [11], although growth of excised root segments of pea is rapidly inhibited even by very low levels of exogenously applied ethylene [5].

Associated with these effects on growth rate, have been the occasional observations of a change in the orientation of growth in response to ethylene [18]. Such an 'ethylene-induced' reorientation of shoot growth, is the basis for the classical triple response exhibited by pea seedlings after exposure to the gas [10, 12].

In this paper we report the observation of ethylene-induced root coiling in tomato seedlings, and discuss the possible origins of this pattern of growth.

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Materials and methods

Growth and ethylene treatment of seedlings

Seeds of *Lycopersicon esculentum* cv Ailsa Craig were used in this investigation. The near isogenic mutant line diageotropica (*dgt*), was obtained from the Glasshouse Crop Research Institute. *Lycopersicon peruvianum* seed was obtained from Dr. C. Rick, University of California, Davis, U.S.A.

Seeds were surface sterilised in 2% sodium hypochlorite for 15 minutes, rinsed twice with distilled water, and then transferred to glass tubes containing 0.3% Ionagar No. 2. These germination tubes were closed with a cotton wool bung, and then incubated at 25 °C in continuous light until the radicle had just penetrated the agar. The tubes were then transferred into desiccators, and maintained in either an atmosphere of ethylene, or an ethylene-free evironment containing a vial of mercuric perchlorate [17]. The ethylene levels in the desiccators were routinely monitored using a Pye Unicam Series 104 gas chromatograph fitted with a hydrogen flame-ionisation detector. Gas samples were separated on an Alumina F_1 column, with analysis temperature and flow rates as described in Ward, Wright, Roberts, Self and Osborne [16].

Agar containing 1 mM silver thiosulphate was obtained by dissolving the agar in a solution of 4 mM sodium thiosulphate and 1 mM silver nitrate mixed in equal volumes. This 'silver agar' was covered with a layer of plain agar to facilitate germination.

Quantification of gravitropic response

Root gravitropic behaviour of tomato seedlings was quantified by the use of a root penetration test. Tomato seeds were germinated in small perspex boxes, with each box containing ionagar of a different concentration. The concentration ranges used varied from 0.3 to 0.8%. After seven days, the number of radicles from normal and *dgt* seedlings that had penetrated the agar surface, were counted. The percentage of radicles penetrating the agar at each concentration gave a measure of the positive gravitropic potency of the two genotypes.

Results

Root coiling time course and ethylene dose: response relationships

After germination, roots of tomato seedlings penetrated the agar and exhibited a strongly positive gravitropic growth habit, elongating at a rate of approximately 10 mm per day. Following exposure to ethylene the growth pattern of the roots changed as a result of the apex constantly altering its orientation to form a highly regular series of helical turns, (Figure 1). The helix invariably decreased in diameter with each successive turn, and could be orientated in either a clockwise or anticlockwise direction.

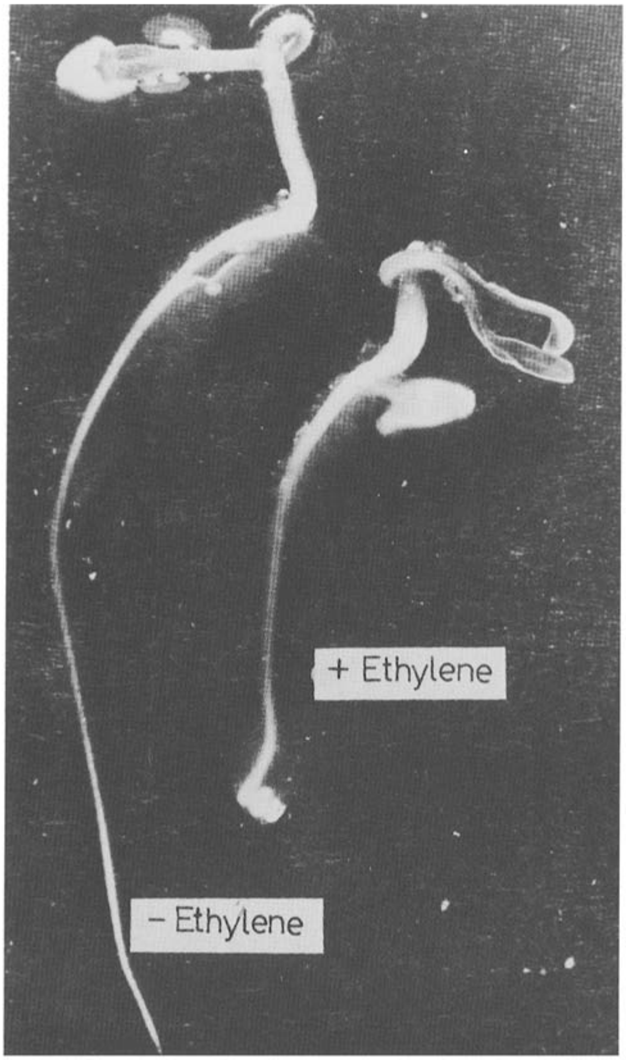

Figure 1. Tomato seedlings grown for 72 h in the absence or presence of C_2H_4 ($10\,\mu l l^{-1}$).

The results of a dose:response experiment showed that with increasing ethylene concentration up to $10\,\mu l\,l^{-1}$, the total number of helical turns produced by each root increased. A further trend observed as a result of exposure to increasing ethylene concentrations, was a progressive decrease in the time required to elicit the first coil.

Roots which had been induced to coil typically ceased growth after about 3 days (Figure 3). These root apices remained viable, and resumed normal vertical growth following transfer to an ethylene-free environment. Prolonged

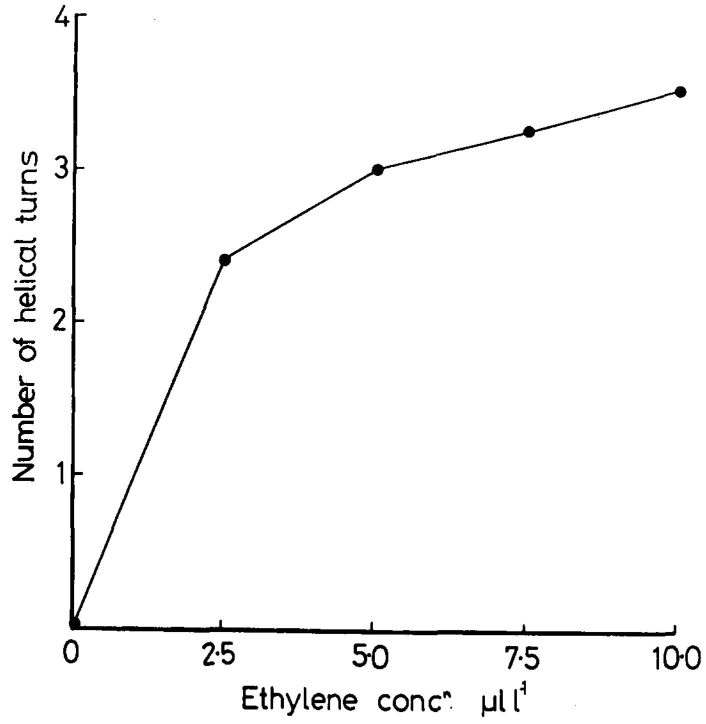

Figure 2. Number of helical turns induced in tomato roots after 72 h exposure to C_2H_4 of increasing concentrations.

exposure to ethylene, resulted in the root apices becoming necrotic followed by the rapid emergence of lateral roots. These laterals also had the capacity to coil.

Incorporation of 1 mM silver thiosulphate into the agar abolished the ethylene-induced root coiling. At this silver concentration, root growth of seedlings incubated in an ethylene-free atmosphere was unimpaired.

Root coiling in tomato mutants and gravitropism

To determine whether root coiling was a unique response of the tomato variety initially selected, seedlings of the wild tomato species *Lycopersicon peruvianum* and a number of mutants of *L. esculentum* were exposed to ethylene. Figure 4 demonstrates that *L. peruvianum* responded in a similar manner as *L. esculentum* to ethylene, and characteristically produced more coils than the cultivated species. All but one of the mutants of *L. esculentum* tested also exhibited the ethylene-induced coiling response, with the rate and extent of coiling being similar to that of the wild type plant. The exception was the mutant genotype diageotropica (*dgt dgt*), which did not change its

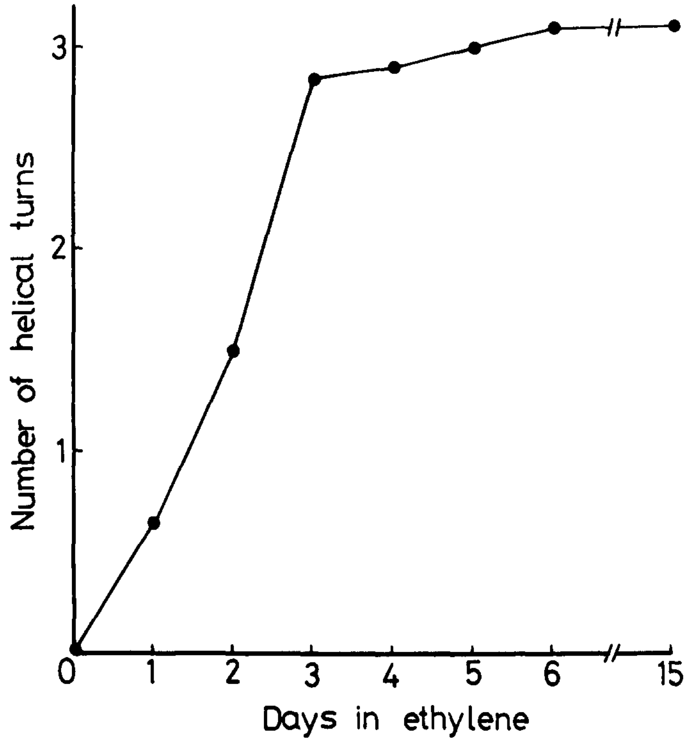

Figure 3. Number of helical turns induced in tomato roots after exposure to C_2H_4 (10 μll^{-1}) for differing periods of time.

root growth pattern following exposure to 10μll^{-1} ethylene (Figure 4). Increasing the ethylene dosage to 25μll^{-1} also failed to elicit a coiling response from this mutant.

The *dgt* mutant gene is known to be associated with defects in root gravitropism [19], however, there is little information available quantifying the extent of the gravitropic abnormality. By germinating seeds of *dgt* and normal types on agar of progressively increasing concentrations, it was possible to quantitatively determine the relative gravitropic ability of the roots. The results of this simple test (Figure 5), and of reorientation experiments, indicate that roots of *dgt* are agravitropic.

Discussion

Exposure of tomato seedlings to ethylene results in the reorientation of the growth of the root apex, and the generation of a tightly packed series of regular coils. This phenomenon has not been previously documented, although Zimmerman [18] did report that treatment of tobacco plants with

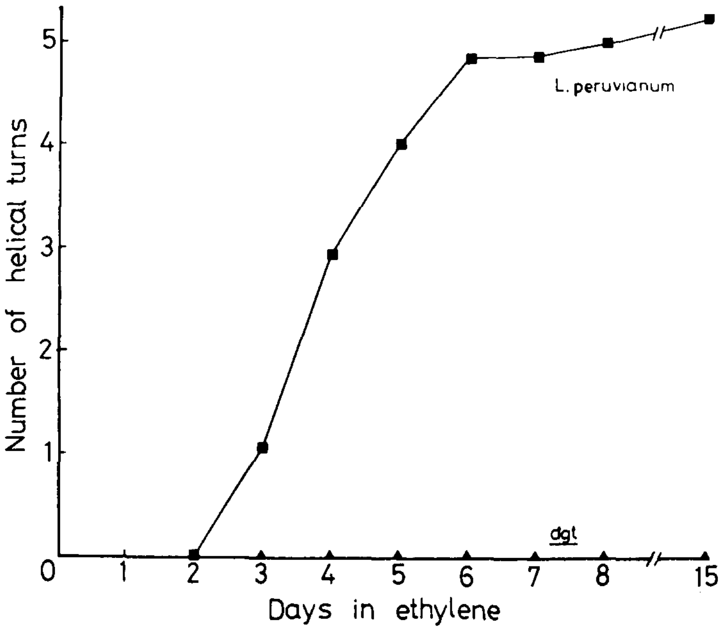

Figure 4. Time course of root coiling of *L. peruvianum* and the mutant diageotropica (*dgt*) in the presence of C_2H_4 ($10\,\mu l l^{-1}$).

0.2% ethylene resulted in the root tips 'turning and twisting'.

Like many other ethylene-induced effects [1], the extent of the response is dependent upon the concentration of ethylene to which the tissue is exposed. As the level of ethylene is raised, the total number of helical turns generated increases and the duration over which they are realised decreases. Extended exposure to the gas leads to a cessation of growth, and ultimately death of the root apex. Further evidence to support the notion that this is a 'typical' ethylene effect is that treatment of the tissue with ethylene in the presence of Ag ions (in the form of silver thiosulphate), abolishes the coiling response. Ag ions are potent inhibitors of ethylene action and antagonise a wide variety of ethylene promoted responses, including senescence, abscission and sex expression [3, 4].

The phenomenon of ethylene-induced root coiling in seedlings is not restricted to the cultivated tomato since the wild tomato species *L. peruvianum* also responds in a similar fashion to the gas. The only tomato seedlings which were unresponsive were homozygous for the mutant allele *dgt.* This observation is of particular interest, since an effect of ethylene on the orientation of shoot growth of *dgt* plants has been described by Zobel [19, 20]. In this case exposure to ethylene has been reported to result in a reorientation of the plant shoot from a diagravitropic to a negatively gravitropic form. No effect of ethylene on the orientation of root growth was documented in accord with the results presented here.

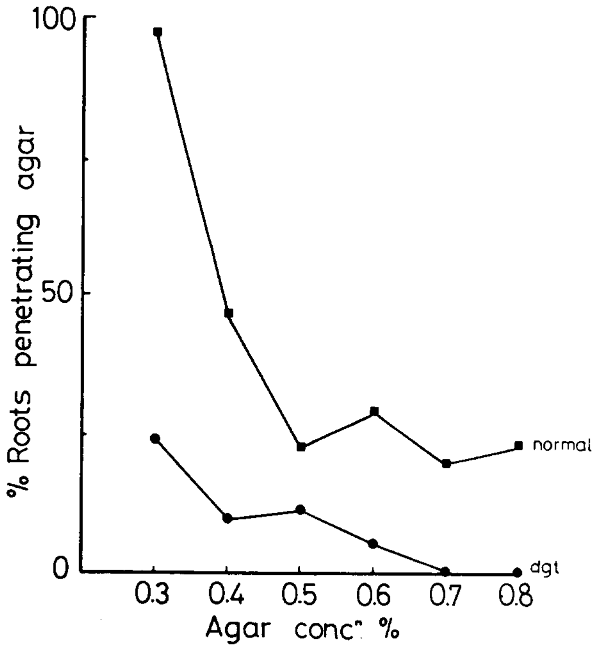

Figure 5. Percentage of primary roots from normal and diageotropica (*dgt*) tomato seedlings which penetrate agar of different concentrations. The higher the percentage of penetrating radicles the greater the gravitropic potency of the seedlings.

In contrast to Zobel's findings, however, our observations indicate that *dgt* roots are not diagravitropic but agravitropic. This inability of *dgt* roots to respond to gravity raises the possibility that a functional gravitropic mechanism is necessary for ethylene-induced root coiling to occur. Chadwick and Burg [5] noted that applied ethylene reversibly abolished the gravitropic response of pea roots. These authors have suggested that endogenous ethylene mediates root gravitropism, and that the normal response mechanism is 'flooded' by exogenous application of this gas. However, the helical coiling of tomato roots cannot simply be regarded as agravitropic behaviour. Indeed if this were so, the agravitropic roots of *dgt* would be expected to coil during normal growth, which is not the case. It is more likely that the coiling response is a continuous series of correction responses which have become disconnected from the gravity stimulus. The mutant roots which cannot respond to gravity, would therefore fail to respond to ethylene.

In some respects the phenomenon of root coiling described in this paper is analagous to tendril coiling. For instance, tendrils coil as a result of a helical growth pattern which may develop in a clockwise or anticlockwise direction (9). This growth form can be induced by ethylene [2, 13], or auxin concentration sufficient to elevate endogenous ethylene production [2, 8]. Increasing levels of ethylene production have also been reported during mechanically induced coiling of pea tendrils [6]. It has been suggested that the contact coiling of tendrils may have its origin in circumnutational movement [7]. Perhaps therefore the ethylene-induced coiling observed in tomato roots is an amplification of the natural circumnutational movement of the root tip.

If this hypothesis was correct, then the agravitropic root of *dgt* might fail to respond to ethylene because it lacks the ability to circumnutate rather than its inability to respond to gravity per se. Certainly a connection between gravitropism and circumnutation has already been reported, since removal of the functional gravitropic system in pea roots, by root tip excision, abolishes circumnutation without preventing elongation [15]. Further work is necessary to elucidate the potentially complex relationships between circumnutation, gravitropism and ethylene-induced root coiling in normal and mutant tomato seedlings.

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