Scientia Horticulturae, 46 (1991) 161–169
Elsevier Science Publishers B.V., Amsterdam

Role of ethylene and bacteria in vascular blockage of cut fronds from the fern *Adiantum raddianum*

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(Accepted for publication 2 July 1990)

ABSTRACT

Van Doorn, W.G., Zagory, D. and Reid, M.S., 1991. Role of ethylene and bacteria in vascular blockage of cut fronds from the fern *Adiantum raddianum*. *Scientia Hortic.*, 46: 161–169.

When placed in water, cut leaves (fronds) of *Adiantum raddianum* Presl. (Tent. Pterid. 1836) wilted within 4 days. Wilting was due to vascular blockage in the basal end of the petiole.

The time to wilting of the fronds was increased by chlorine bleach, silver nitrate and cobaltous ions. Exogenous ethylene, l-aminocyclopropane-l-carboxylic acid (ACC) or ethephon had no effect on time to wilting. Inhibitors of ethylene synthesis (aminoethoxyvinylglycine (AVG)) or ethylene action (silver thiosulphate (STS)) also had little effect. Aminooxyacetic acid (AOA) had no effect when buffered at pH 7, but extended time to wilting when in unbuffered solutions (pH ~ 3). Citric acid (pH ~ 3) also increased time to wilting. Whenever time to wilting was increased, the number of bacteria in the basal end of the petioles was lower than in controls. A mixed population of bacteria originating from the petioles reduced time to wilting. The experiments indicate that ethylene played no role in vascular blockage. The occlusion was apparently solely related to the presence of bacteria.

Keywords: *Adiantum raddianum* Presl.; bacteria; cut fronds; ethylene; vascular occlusion

Abbreviations: ACC = l-aminocyclopropane-1-carboxylic acid; AOA = aminooxyacetic acid; AVG = aminoethoxyvinylglycine; cfu = colony-forming units; DI = deionized; HQC = 8-hydroxyquinoline citrate; STS = silver thiosulphate.

INTRODUCTION

Leaves of the fern *Adiantum raddianum* Presl. (Tent. Pterid. 1836), wilt within a few days after cutting (Fujino et al., 1983a). The time to wilting of the leaves (usually called fronds) could be significantly delayed by including silver nitrate in the vase solution (Fujino and Reid, 1983; Fujino et al., 1983a). As silver ions are known to inhibit the effects of ethylene (Beyer, 1976), it was suggested that the effect of silver nitrate was related to a wound reaction mediated by ethylene. After cutting, the basal end of the petioles produced considerable amounts of ethylene (Fujino and Reid, 1983). Exoge-

0304-4238/91/$03.50

nous ethylene has been found to induce vascular blockage in *Ricinus communis* (VanderMolen et al., 1983). The hypothesis that frond wilting was mediated by ethylene was strengthened by the fact that aminooxyacetic acid (AOA) and cobaltous ions, known to inhibit the synthesis of ethylene, also delayed the onset of wilting (Fujino and Reid, 1983).

Silver nitrate and cobaltous ions are also known to have antibacterial properties. A number of other antibacterial chemicals such as 8-hydroxyquinoline citrate (HQC) and Physan-20 (a mixture of quaternary ammonium compounds), however, did not delay wilting. This suggested that the effect of silver nitrate and cobaltous ions was not related to their antibacterial properties (Fujino and Reid, 1983).

We re-evaluated these findings after preliminary tests with cut rose flowers had indicated that AOA had an antibacterial effect, possibly as a result of the low pH of the solution, and that some antibacterial chemicals had a toxic effect on cut rose flowers, resulting in early wilting.

MATERIALS AND METHODS

Plant material. – Plants of the fern *A. raddianum* were grown under 25% shade in the greenhouse of the Department of Experimental Horticulture (University of California, Davis) at 25/18°C day/night temperature.

Newly mature fronds were randomly cut at the base of the petioles (Ogura, 1972) and petiole ends were immediately placed in deionized (DI) water. The petioles were placed in individual test solutions within 10 min.

The fronds were held at 22 ± 2°C and 50 ± 7% relative humidity; at a photon flux density (photosynthetically active radiation of 15 μmol m^{-2} s^{-1} for 12 h per day, provided by cool white fluorescent tubes. Solutions were placed in sterile plastic bags (liners), which were held inside plastic tubes. The temperature of the solutions was 22 ± 2°C.

The time between placing the fronds in solutions and the first appearance of wilting (desiccation of the pinnae) was measured in 6–8 replicate fronds. In some experiments, ~30 mm was cut (under water) from the basal end of the petioles when frond wilting started. The treatment differences were compared by analysis of variance and *F*-tests.

Chemicals. – Chemicals were obtained from Sigma (St. Louis, MO) except silver nitrate (Mallinckrodt, Paris, KT), HQC (Merck, Ratway, NY), ethephon (Florel; from Amchem, Ambler, PA), Physan-20 (Consan Pacific, Whittier, GA) and chlorine bleach (All Pure, Tracy, CA).

Buffered AOA was made by preparing a pH 7.0 solution of Hepes (3570 mg l^{-1}) by adding KOH, then adding the desired AOA concentration. Silver thiosulphate (STS) was made by adding a solution of silver nitrate to a solu-

tion of sodium thiosulphate. The concentration of sodium thiosulphate was eight times that of silver nitrate.

Exogenous ethylene. – Ethylene treatments were given by placing fronds in DI water in glass tanks ventilated with air ($\sim 30\ l\ h^{-1}$) or air containing ethylene (0.3, 3 and 20 $\mu l\ l^{-1}$). The air containing ethylene was passed through the solution in which the petioles were placed before it entered the headspace of the tank. The ethylene concentration in the headspace was continuously measured by automatic photoionization gas chromatography (Photovac 10S30). The relative humidity in the tanks was ~90%.

pH. – The pH of the solutions was determined at the onset of the study, using a Corning pH-meter and a 125 Corning combination X-EL electrode. Four vases were used for each determination.

Bacteria. – After 7 days, the fronds were taken out of the solution and 7 cm from the basal end of the petiole was sterilized by careful blotting with ethanol (98% v/v). Five centimeters were cut from the basal end with standard sterile equipment and further cut into segments of ~2 mm. These segments were placed in pre-sterilized glass tubes containing sterile DI water. After vortexing for 1 min, the extracts were diluted 10-fold with sterile DI water. The diluted extracts were spread on Kings B agar using a spathula and the agar plates were incubated at 25 °C for 2 days. Three stems of each treatment were analyzed.

The mixed bacterial population was isolated from petioles of fronds held in DI water for 7 days. Bacteria were grown on agar, and colonies were scraped off, placed in water and their titre was adjusted to 10^4 colony-forming units (cfu) l^{-1}. The petioles of freshly cut fronds were placed in this solution to study the effect of bacteria on time to wilting.

RESULTS

Wilting of cut fronds and frond senescence on the plant. – Frond senescence in intact plants occurs at least 3–4 weeks after the frond unfolds. It is characterized by gradual yellowing, followed by desiccation. In cut fronds placed in DI water, no leaf yellowing was observed, but the pinnae wilted irreversibly within 3–6 days. When the ambient relatively humidity was increased, the time to wilting increased (Table 1). When the stems were recut at the first symptoms of wilting, a temporary recovery of turgor was observed. The symptoms observed in cut fronds placed in DI water were also observed in intact plants allowed to dry.

Effects of ethylene and ethylene-releasing substances. – Various concentrations of ethylene had no effect on the time to wilting of the pinnae (Table 1).

TABLE 1

Effects of ethephon, ACC and ethylene on time to wilting in cut fronds of *A. raddianum*

	Time to wilting (days)	Number of bacteria in petioles (cfu in the basal 5 cm)
Ethephon (RH 50%) ($mg\,l^{-1}$)		
DI control	2.8 ± 1.4	$4–7\times10^4$
50	2.7 ± 1.7	$5–6\times10^4$
100	2.9 ± 1.6	$3–5\times10^4$
ACC (RH 50%) ($mg\,l^{-1}$)		
DI control	2.8 ± 1.4	$2–5\times10^4$
50	2.9 ± 1.7	$3–4\times10^4$
100	2.8 ± 1.6	$2–8\times10^4$
Ethylene (RH 90%) ($mg\,l^{-1}$)		
DI control	7.2 ± 0.5	$5–9\times10^4$
0.3	7.0 ± 0.6	$3–6\times10^4$
3	7.4 ± 0.5	$2–7\times10^4$
20	7.3 ± 0.8	$6–8\times10^4$

RH = relative humidity.
Time to wilting is the mean of six replications ± S.D.
The number of bacteria refers to the range found in three stems. Petioles were dipped in an aqueous ethephon solution for 30 s, kept in a solution containing ACC or in a solution in which ethylene was passed continuously.

1-Aminocyclopropane-l-carboxylic acid (ACC) applied to the vase solution or dipping of the petioles in ethephon similarly had no effect on wilting (Table 1). None of these treatments affected the total number of bacteria in the basal 5 cm of the petioles (Table 1).

Effects of inhibitors of ethylene synthesis. – Aminoethoxyvinylglycine (AVG) was found to have no effect on time to wilting, nor on the number of bacteria in the petioles (Table 2). AOA lowered the pH, reduced the number of bacteria and delayed the onset of wilting. When buffered in Hepes at pH 7.0, AOA had no effect on the number of bacteria, nor on wilting. Cobaltous nitrate at 185 and 290 $mg\,l^{-1}$ reduced the number of bacteria in the petioles and delayed the onset of wilting (Table 2).

Effect of inhibitors of ethylene action. – Silver, when applied as a pre-treatment of STS, had no effect on time to wilting, but silver nitrate considerably delayed the onset of wilting (Table 2). STS did not have an effect on the number of bacteria in the petioles, while silver nitrate reduced the number to zero (Table 2).

TABLE 2

Effect of inhibitors of ethylene synthesis and inhibitors of ethylene action on time to wilting in cut *A. raddianum* fronds, on the pH of the vase solution on Day 0 after treatment and the number of bacteria in the basal 5 cm of the petioles 7 days after treatment.

	Time to wilting (days)	pH of the vase solution	Number of bacteria in petioles (cfu in the basal 5 cm)
DI control	4.3±1.2	5.6±0.1	$3–8\times10^4$
AVG ($mg\,l^{-1}$)			
10	4.6±1.1	5.6±0.1	$1–4\times10^4$
20	4.1±1.1	5.6±0.2	$8–13\times10^4$
40	3.8±0.6	5.6±0.1	$2–10\times10^4$
AOA ($mg\,l^{-1}$)			
27	5.4±1.3	3.3±0.0	$2–6\times10^4$
54	6.8±1.7***	3.1±0.1	0***
108	8.8±2.2***	2.9±0.1	0***
216	10.6±2.0***	2.8±0.0	0***
AOA ($mg\,l^{-1}$)+Hepes (3570 $mg\,l^{-1}$)			
Hepes-control	4.1±1.0	7.0±0.0	$3–8\times10^4$
27	4.6±1.8	7.0±0.0	$2–6\times10^4$
54	4.7±1.6	7.0±0.0	$1–4\times10^4$
108	4.6±1.2	6.9±0.1	$1–3\times10^4$
216	4.8±1.5	6.8±0.1	$1–3\times10^4$
Cobaltous nitrate ($mg\,l^{-1}$)			
92	3.5±0.5	5.7±0.1	$1–7\times10^4$
185	6.7±1.4***	5.5±0.0	0***
290	5.7±3.0***	5.4±0.1	0***
580	3.5±1.1	5.1±0.2	0***
STS ($mg\,l^{-1}$)			
656, 4 h	4.2±1.0	5.6±0.1	$3–4\times10^4$
1312, 4 h	4.0±1.3	5.6±0.2	$3–8\times10^4$
2624, 4 h	4.4±1.0	5.6±0.1	$1–6\times10^4$
Silver nitrate ($mg\,l^{-1}$)			
12.5	10.6±3.2***	5.3±0.1	0***
25.0	12.7±3.0***	4.9±0.2	0***

***Significantly different from the control ($P>0.01$). Time to wilting is the mean of six (cobaltous nitrate) or eight replications ± S.D. The number of bacteria refers to the range found in three stems.

Effect of antibacterial chemicals. – Most of the chemicals tested were toxic to fronds of maidenhair ferns. HQC and Physan-20 resulted in browning of the pinnae, starting along the vascular bundles and at the base of the pinnae. This

was followed by wilting (Table 3). Aluminium sulphate and citric acid resulted in browning of the pinnae which started at the top, also leading to early wilting. The development of a bacterial population in the basal 5 cm of the petioles was inhibited by aluminium sulphate, HQC, Physan and chlorine bleach. Aluminium sulphate and Physan-20 did not delay the onset of wilting,

TABLE 3

Effect of antibacterial compounds and bacteria on time to wilting in cut *A. raddianum* fronds, pH of the vase solution on Day 0 after treatment and the number of bacteria in the basal 5 cm of the petioles 7 days after treatment. The number of bacteria in the treatment in which bacteria were added was determined after 2 days

	Time to wilting (days)	pH of the solution	Number of bacteria in petioles (cfu in the basal 5 cm)
DI control	3.4±0.8	5.6±0.1	$3–8\times10^4$
Aluminium sulphate ($mg\,l^{-1}$)			
230	4.4±1.1	4.2±0.0	$2–5\times10^4$
460	4.5±1.5	4.0±0.0	$4–6\times10^4$
920	4.0±1.5	3.9±0.0	0***
8-Hydroxyquinoline citrate ($mg\,l^{-1}$)			
250	6.0±1.6***	3.8±0.0	0***
500	8.0±4.5***	3.6±0.0	0***
1000	2.5±0.5	3.5±0.0	0***
Physan ($mg\,l^{-1}$)			
50	3.3±0.5	5.6±0.2	0***
100	3.4±0.5	5.6±0.3	0***
200	5.8±2.8	5.6±0.2	0***
400	5.5±2.8	5.6±0.1	0***
Chlorine bleach ($mg\,l^{-1}$)			
10	18.4±4.3***	8.4±0.2	0***
20	20.0±3.8***	8.8±0.3	0***
Citric acid ($mg\,l^{-1}$)			
60	5.8±2.6	3.1±0.0	nd
120	9.1±3.1***	2.9±0.0	nd
240	9.7±2.5***	2.8±0.0	nd
480	8.8±2.6***	2.6±0.0	nd
Bacteria			
10^4 cfu ml^{-1}	1.2±0.3	6.9±0.3	$4–9\times10^4$

nd: not determined.

***Significantly different from the control ($P>0.01$). Time to wilting is the mean of seven (citric acid) or eight replications ± S.D. The number of bacteria refers to the range found in three stems.

while HQC or citric acid delayed wilting. Pinnae wilting was, however, much delayed by chlorine bleach.

A mixed bacterial population, originating from petioles of fronds held in DI water for 7 days, reduced time to wilting (Table 3).

DISCUSSION

Wilting symptoms in cut fronds were apparently due to a low water potential in the tissue, as the symptoms were delayed by maintaining a high ambient relative humidity. During vase life, a blockage which limited water uptake apparently developed in the basal end of the petioles since a temporary regaining of turgor was observed when the petioles were recut. The blockage coincided with a high number of bacteria in the basal 5 cm of the petiole.

Development of a low turgor in the pinnae may be related to the anatomy of the petioles. The petioles of *A. raddianum* contained only ~40 water-conducting elements, as indicated by examining transverse sections with a light microscope. In our experiments, the leaf area of the fronds was 100–210 cm^2. Rose stems are also easily occluded, which gives rise to a low water potential in the leaves (De Witte and Van Doorn, 1988). Rose stems were found to contain ~1600 water-conducting vessels (Van Doorn, 1986) of about the same diameter as those in the fern petioles, and a leaf area of ~500 cm^2. Hence, compared with cut rose flowers, the ratio of water-conducting area to transpirational area is relatively small in *A. raddianum*.

The hypothesis of Fujino and Reid (1983), that vascular blockage of cut fronds of *A. raddianum* is due to ethylene, was mainly based on the evidence that antibacterial compounds such as HQC, citric acid and Physan did not have the same positive effect as silver nitrate. It was hard to distinguish, however, between antibacterial effects and ethylene effects since a number of chemicals that interfere with the synthesis or action of ethylene, such as silver nitrate, cobaltous ions and AOA, also have antibacterial properties.

It is now shown that the absence of an effect of antibacterial compounds such as HQC, citric acid and Physan may relate to a negative effect on the leaves, as indicated by discoloration. At the concentration tested, chlorine bleach (sodium hypochlorite) did not have an effect on leaf colour and considerably delayed time to wilting. Both chlorine bleach and silver nitrate prevented the development of a bacterial population in the petioles.

The hypothesis that ethylene is the cause of the blockage found in the petioles of *A. raddianum* was not substantiated. Ethylene, ethylene-releasing substances and AVG, an inhibitor of ethylene synthesis (Lieberman, 1979), had no effect on frond wilting. In addition, the effect of AOA, another potent inhibitor of ethylene biosynthesis (Yang, 1980) disappeared when the pH of the vase solution was kept close to neutral.

When silver was applied as the nitrate salt, it had antibacterial properties

and delayed the onset of wilting. When silver was applied as STS, which had no antibacterial properties, time to wilting was not affected.

The close relationship between the absence or low numbers of bacteria and a long vase life suggests that bacteria were the cause of the vascular blockage in the cut fronds. This hypothesis was supported by the effect of exogenously applied bacteria on time to wilting.

Zagory and Reid (1986) showed that some bacteria from vase water of carnation flowers produced ethylene. Whatever the mechanism of action of bacteria in inducing vascular blockage in cut fronds may be, the above evidence against ethylene as the inducer of vascular blockage would also apply to ethylene produced by bacteria.

In a scanning electron microscope study, Fujino et al. (1983b) found an amorphous substance in xylem vessels of the petioles of *A. raddianum*. The amorphous substance was interpreted to be released into the xylem by parenchyma cells. Its presence may also relate to microorganisms. One possibility is that bacteria induce the parenchyma cells to deposit the plugging substance. Many of the bacteria found in vase water, however, are known to produce copious amounts of slime (De Witte and Van Doorn, 1988). Bacterial slime, which consists mainly of linear polysaccharides (Kennedy and Sutherland, 1987), is released into the vase solution and will be taken up by the petioles. The amorphous plugs found in the xylem elements of cut fronds may, therefore, consist of these exopolysaccharides. Bacteria are too large to pass through the pores in the walls of water-conducting elements (Van Doorn et al., 1989), while a fraction of the polysaccharide molecules may pass, giving rise to xylem elements containing the amorphous polysaccharide only.

The present experiments indicate, therefore, that ethylene , as a result of a wound reaction after cutting, is not triggering vascular blockage in cut fronds of *A. raddianum*. The correlation of wilting with a high number of bacteria in the petioles, and the effect of exogenous bacteria, suggest that the blockage is due to the presence of bacteria.

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