FLOODING RESISTANCE AND ETHYLENE. II. APPLICATION OF AN ADVANCED LASER-DRIVEN PHOTOACOUSTIC TECHNIQUE IN ETHYLENE MEASUREMENTS ON FLOODED *RUMEX* PLANTS.

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Flooding is an extremely severe stress condition for many higher plants. Species which occur in frequently submerged or waterlogged habitats often show a number of morphological and anatomical adaptations, which avoid the hazard of hypoxia or anoxia during a prolonged period of flooding. Common examples of these adaptations are petiole and stem elongation [5], which enable the submerged leaves to reach the water surface, and the development of aerenchymatous tissues, through which atmospheric oxygen can be transported from the emerging leaf tips to the submerged parts of the plant [2].
In some species of the genus *Rumex* the accelerated growth of submerged petioles starts within hours after the onset of flooding (fig. 1A). Ethylene is found to accumulate in the shoot during submergence [1,2] and has a strong promotive effect on petiole elongation (fig. 1B, [5]).

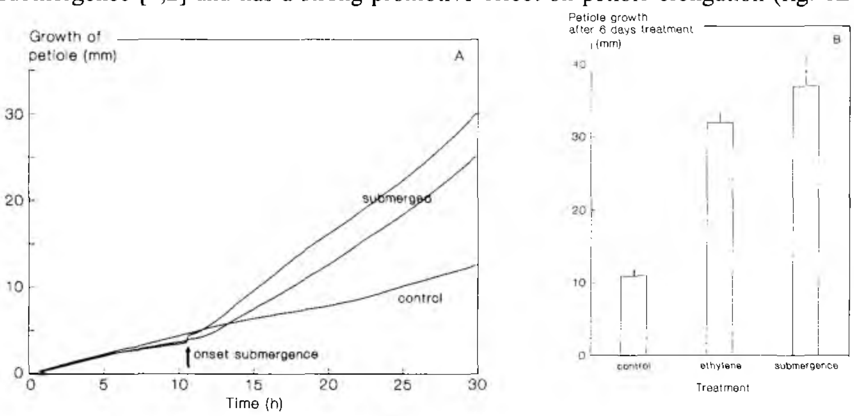

Fig. 1A. Time course of elongation of young petioles of *R. palustris* upon submergence. Upper lines: submerged; lower line: drained control. Age of the plants was 26 days; B. Elongation of young petioles of *R. palustris* upon submergence or exposure to 5 ppm ethylene for 6 days. Age of the plants was 26 days; n=9; bars represent SE.

Oxygen levels in the flooded soil decrease within a few hours to hypoxic or even anoxic levels, mainly as a result of the high oxygen consumption rate of microorganisms and plant roots. In response to this limited soil oxygen availability, flooding-adapted *Rumex* species develop a large number of highly porous, adventitious roots [2], which start to emerge from the cortex of the tap root within one or two days after the onset of flooding (fig. 2A). Besides other hormones,

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ethylene appears to be involved in the regulation of adventitious root formation, since application of ethylene to aerated root systems of plants on hydroculture caused adventitious root formation to a comparable extent as under low oxygen concentrations (fig. 2B).

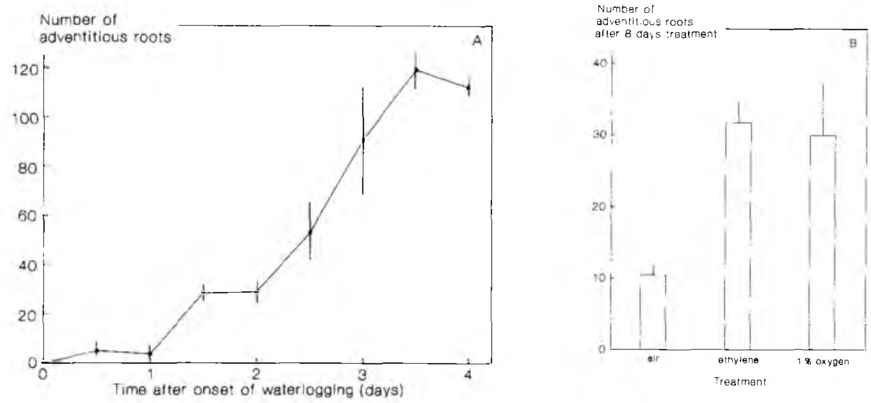

Fig. 2A. Time course of adventitious root formation of *R. palustris* upon waterlogging. Age of the plants was 70 days; n= 4; bars represent SE; B. Adventitious root formation of *R. palustris* in hydroculture after 8 days of exposure to hypoxic conditions (1% oxygen) or ethylene (10.0 ppm). Age of the plants was 49 days; n= 4; bars represent SE.

The current methods of ethylene detection all depend on accumulation of the gas, which gives only a global insight into the timing of the mentioned ethylene mediated processes in *Rumex*. Therefore, a technique is required which can detect the very low concentrations of ethylene, released by a plant in the gas flow of a flow-through system. Furthermore, measurements should succeed each other within minutes or even seconds to get a most accurate view of the timing of a process. A technique, which depends on the photoacoustic principle has shown to overcome both problems [3].

The photoacoustic effect occurs when a frequently interrupted light beam, in this case a laser beam, hits a gas sample. At an appropriate laser wave length, ethylene molecules will be excitated by the light. Due to this excitation, the kinetic energy of the gas molecules increases during every light pulse, and decreases during the interruption of the beam, thus creating small pressure changes. These pressure changes are recorded as acoustic waves. The concentration of ethylene in the sample flow is reflected in the amplitude of the signal.

In this way, we have achieved a sensitivity of 0.03 ppb ethylene under average experimental conditions. In addition, measurements can be made every minute. This method made it possible to establish the exact time course of ethylene evolution from the shoot during de-submergence, while at present ethylene production of flooded roots is under investigation.

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