The Role of Ethylene in the Induction of Apogamous Buds in *Pteridium* Gametophytes

Harold W. Elmore and Dean P. Whittier
Department of General Biology, Vanderbilt University,
Nashville, Tennessee 37235, U.S.A.

Received December 5, 1972/January 10, 1973

Summary. The level of induced apogamy in gametophytic colonies of *Pteridium aquilinum* (L.) Kuhn is altered by varying the number of colonies per culture vessel or by including a constant number of colonies in culture vessels of different volumes. In either case, placing vials of mercuric perchlorate, an absorber of ethylene, within the closed culture vessels reduced the apogamous response to a very low level. Production of ethylene by the gametophytes was demonstrated by gas chromatography. Ethylene supplied in a continuous-flow system promoted the apogamous response above that of an air control.

Introduction

Apogamy, the organization of sporophytic plants from gametophytic tissue without syngamy, can be induced in *Pteridium* gametophytes if the nutrient medium is supplemented with sugar, and its level can be controlled by varying the sugar concentration (Whittier and Steeves, 1960; Whittier, 1964). However, the regulatory factors governing the induction of apogamy remained unknown.

Since experiments on induced apogamy utilizing sealed containers gave large apogamous responses (Whittier and Pratt, 1971), it seemed possible that the gametophytes released a volatile which influenced apogamy. Ethylene was hypothesized to be this volatile because it is produced by a wide range of plants and plant tissues and because it causes a large number of different responses in plants (Burg, 1962). The present investigation was undertaken to determine if ethylene had any influence on the induction of apogamy in gametophytes of *Pteridium aquilinum* (L.) Kuhn.

Materials and Methods

The gametophytes, the nutrient medium, and the sterile culture techniques were the same as used by Whittier and Pratt (1971). The gametophytes were grown under continuous illumination at 1250 lux (Gro-lux fluorescent lamps; Sylvania, Salem, Mass., U.S.A.) and at a temperature of 24 ± 1°C. The nutrient medium that was used in all experiments contained 4% sucrose. Pieces of gametophytes weighing
ca. 0.1 g were taken from stock cultures to initiate the experiments. After 36 days the apogamous sporophytes were counted and the fresh weights of the colonies recorded. The apogamous response has been expressed as apogamous plants per gram of gametophytic tissue. Since ethylene did not affect the final weight of the gametophytic colonies, expressing the results in this way gave an accurate measure of the response. The data were analyzed by the analysis of variance and Duncan’s multiple range test (Li, 1964) to determine statistically significant differences.

The effect of endogenous ethylene on apogamy was studied in two experiments. In the first, 1, 5, 9 or 12 gametophytic colonies were sealed in 500-ml Pyrex storage dishes containing 200 ml of the nutrient medium. In the second experiment, 3 gametophytic colonies were grown in 200-ml glass bottles containing 50 ml of the nutrient medium, and each bottle was then enclosed in 0.5-, 1.0- or 2.0-l jars. In both experiments loss of ethylene was minimized by sealing the glass lids onto the culture vessels with 3 layers of parafilm. Glass vials containing 3 ml per gametophytic colony of mercuric perchlorate, an active absorber of ethylene (Young et al., 1952), were included in the control vessels.

Ethylene production by the gametophytes was measured in the following manner. Gametophytes which had been grown for 1 week on the medium with agar were transferred to 50-ml flasks containing 4 ml of the medium without agar. After 1 h the flasks were closed with neoprene stoppers fitted with serum caps, and were placed under the normal culture conditions. To determine the rate of ethylene production gas samples were withdrawn from the flasks at intervals over a 24-h period and were analyzed with an Aerograph 200 flame-ionization gas chromatograph equipped with an aluminum-oxide column.

A continuous-flow system similar to the one described by Pratt et al. (1960) was used to study the effects of exogenous ethylene on the apogamous response. The gametophytes, contained in storage dishes with loosely fitting lids, were grown in the continuous-flow system with controlled concentrations of ethylene.

**Results**

Growing various numbers of gametophytic colonies in the 500-ml culture vessels affected the apogamous response (Fig. 1). Maximum induction of apogamy was obtained with 5 colonies per dish. With more than 5 colonies per culture vessel the response decreased toward the level with one colony per dish. When 3 gametophytic colonies were grown in glass jars of various volumes (Fig. 2), maximum induction of apogamy took place in the smallest (0.5-l) culture vessels, and the response decreased with increasing volume of the culture vessels. In either type of experiment, inclusion of mercuric perchlorate within the culture vessels reduced the induction of apogamy to a level below the lowest level without mercuric perchlorate and eliminated any statistically significant variation in the response to the changes in number of gametophytes and volume of culture vessel (Figs. 1, 2).

The results of the preceding experiments suggested that an active volatile was produced by the gametophytes, and that this volatile was ethylene. Flame-ionization gas chromatography was employed to demonstrate ethylene production by *Pteridium* gametophytes. Over a 24-h period, the rate of ethylene production by the gametophytes remained