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# An *in vitro* model to study abscission in grapevine (*Vitis vinifera* L.)

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Key words: abscission, *Vitis vinifera* L., *in vitro* model, ethylene, coulure.

**Introduction:** In grapevine, abscission is mainly related to young fruit. Bessis and Fournioux (1992) have demonstrated that berry drop begins at bloom and ends two weeks after flowering. Abscission occurs at the basis of the pedicel, after the formation of an abscission zone. Many factors influence berry drop, *e.g.* variety, weather at flowering, competition between vegetative and reproductive organs (Carbonneau and Ollat 1993).

To facilitate studies on the grapevine pedicel abscission zone, a model was established using young fruit in Petri dishes (Bessis and Fournioux 1992, Bessis *et al.* 2000). However, the short period of flowering restricted the application of this model. Better knowledge on the mechanisms of abscission requires a model which could be used all year long.

The aim of this paper is to present a model for grapevine abscission studies on the basis of leaf abscission of *in vitro*-cultivated grapevine. The possibility to extrapolate the results obtained to floral abscission is discussed.

**Material and Methods:** Plant material: Grapevine microcuttings (*Vitis vinifera* L., cv. Chardonnay, clone 76), were propagated *in vitro* on a medium described by Fournioux and Bessis (1993). After a culture cycle of 80 d, the shoots of the plantlets were sectioned in one-leaf cuttings and the blade was removed, *i.e.* the nodal explants obtained consisted of a section of shoot bearing a petiole. An abscission zone was located at the shoot-petiole junction. 100 explants were planted in culture chambers as described below.

Experimental system: Abscission was induced in air-tight polymethylmethacrylate culture containers (25 x 16 x 7 cm). Explants were planted out in an agarose gel in water. Thereafter, culture containers were closed and ethylene (L'Air Liquide, Paris, France) was injected through a septum situated on top of the container by a chromatographic syringe. Its concentration in the containers was checked by gas chromatography.

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chamber at 19 °C. Air was replaced by opening the containers at 12-h-intervals. During these aerations, the rate of abscission was estimated by exerting a slight pressure on the explant's petiole. Then, the container was closed and ethylene was injected again into the container.

Statistical analysis: Experiments were repeated 6 times. Means of the abscission rate after 48 and 84 h were used for statistical analysis. Means obtained for each ethylene treatment were compared two by two with the Student-Fischer's *t*-test ($p$ = 0.05 and 0.01).

**Results and Discussion:** Petiolar abscission of explants was induced by ethylene. For all tested ethylene concentrations, a significant abscission was observed only 36 h after planting (Figure). 100 % abscission of explants was obtained after 96-120 h. Even very small ethylene concentrations ($10^{-2}$ µl·l$^{-1}$) induced abscission. These results agree with observations indicating that physiological ethylene effects can be obtained with exogenous levels between $10^{-2}$ and 1.0 µl·l$^{-1}$ (Abeles *et al.* 1992). Up to $10^{3}$ µl·l$^{-1}$, the abscission curve rises sharply and is dependent on ethylene concentration. Statistical treatment confirmed the relation between the abscission rate and ethylene concentration (Table). Beyond $10^{3}$ µl·l$^{-1}$, no correlation was observed and 100 % abscission was obtained only after 96 h. Without ethylene and in spite of leaf removal, rates of petiolar abscission were very small. This lack of reaction persisted for several weeks. This is unusual since leaf removal has often been used to produce petiolar abscission (Levis and Varner 1970, Rascio *et al.* 1987). The anomaly could be related to the juvenile caracters of *in vitro* grapevine (Fournioux and Bessis 1986, 1993). Thus, our basic idea to finalize the model was to induce abscission using another grapevine organ whose abscission zone should be easy to stimulate. Leaf petioles of *in vitro* plantlets are useful objects. *In vitro* culture enables to obtain physiologically homogeneous plant material, regularly and in high quan-

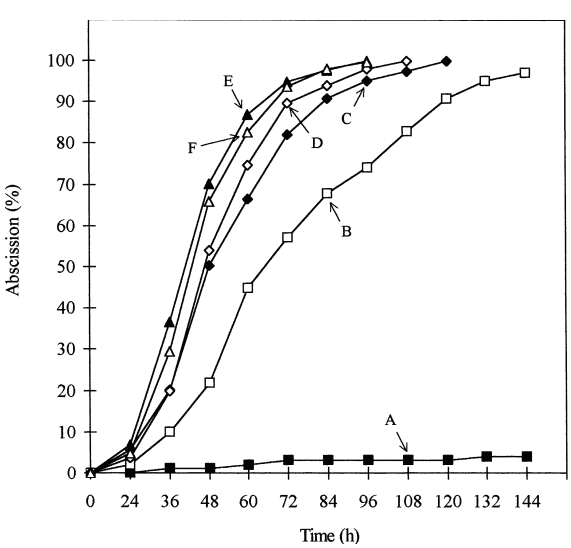


Figure: Effect of various ethylene concentrations (µl·l$^{-1}$) on the time course of petiolar abscission. **A**: control without ethylene; **B**: $10^{-2}$, **C**: $10^{-1}$, **D**: 10, **E**: $10^{3}$, **F**: $10^{4}$.

T a b l e

Means of abscission *vs.* ethylene concentration ($\mu l \cdot l^{-1}$) 48 and 84 h after ethylene treatment. Values are means of 6 replicates. * indicates significant differences between treatments. *: $p = 0.05$, **: $p = 0.01$, NS: no significance, nc: not calculated

| 48 h | Control | $10^{-2}$ | $10^{-1}$ | 10 | $10^{3}$ | $10^{4}$ |
|---|---|---|---|---|---|---|
| | 1 % | 22.4 % | 50.0 % | 54.2 % | 70.3 % | 65.7 % |
| Control | | | | | | |
| $10^{-2}$ | nc | | | | | |
| $10^{-1}$ | nc | nc | | | | |
| 10 | nc | nc | * | | | |
| $10^{3}$ | nc | ** | ** | ** | | |
| $10^{4}$ | nc | nc | ** | ** | NS | |

| 84 h | Control | $10^{-2}$ | $10^{-1}$ | 10 | $10^{3}$ | $10^{4}$ |
|---|---|---|---|---|---|---|
| | 3 % | 67.5 % | 91.2 % | 94.3 % | 97.9 % | 97.3 % |
| Control | | | | | | |
| $10^{-2}$ | nc | | | | | |
| $10^{-1}$ | nc | nc | | | | |
| 10 | nc | nc | * | | | |
| $10^{3}$ | nc | nc | ** | * | | |
| $10^{4}$ | nc | nc | ** | NS | NS | |

tity. Exogenous ethylene stimulation to obtain abscission of the petiole allows to make a precise control of abscission.

Moreover, we were able to counteract ethylene with auxin, silver thiosulfate, calcium (HILT, unpubl.). These data suggest that our *in vitro* model agrees with general processes of abscission described by SEXTON (1995) and BROWN (1997).

**Conclusion:** Our *in vitro* model will allow us to make precise studies on the mechanisms of abscission. However, the possibility to extrapolate the results obtained from this model to young fruit abscission remains hypothetical. To validate the relation between *in vitro* petiolar abscission and floral abscission, it would be interesting to compare the ultrastructure of both zones and to analyze the enzymatic profile of both zones during abscission, as abscission is caused by synthesis of specific hydrolases isozymes (BROWN 1997).

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