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The two combined intransit flux reduction-properties of the grape marc extract

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Introduction

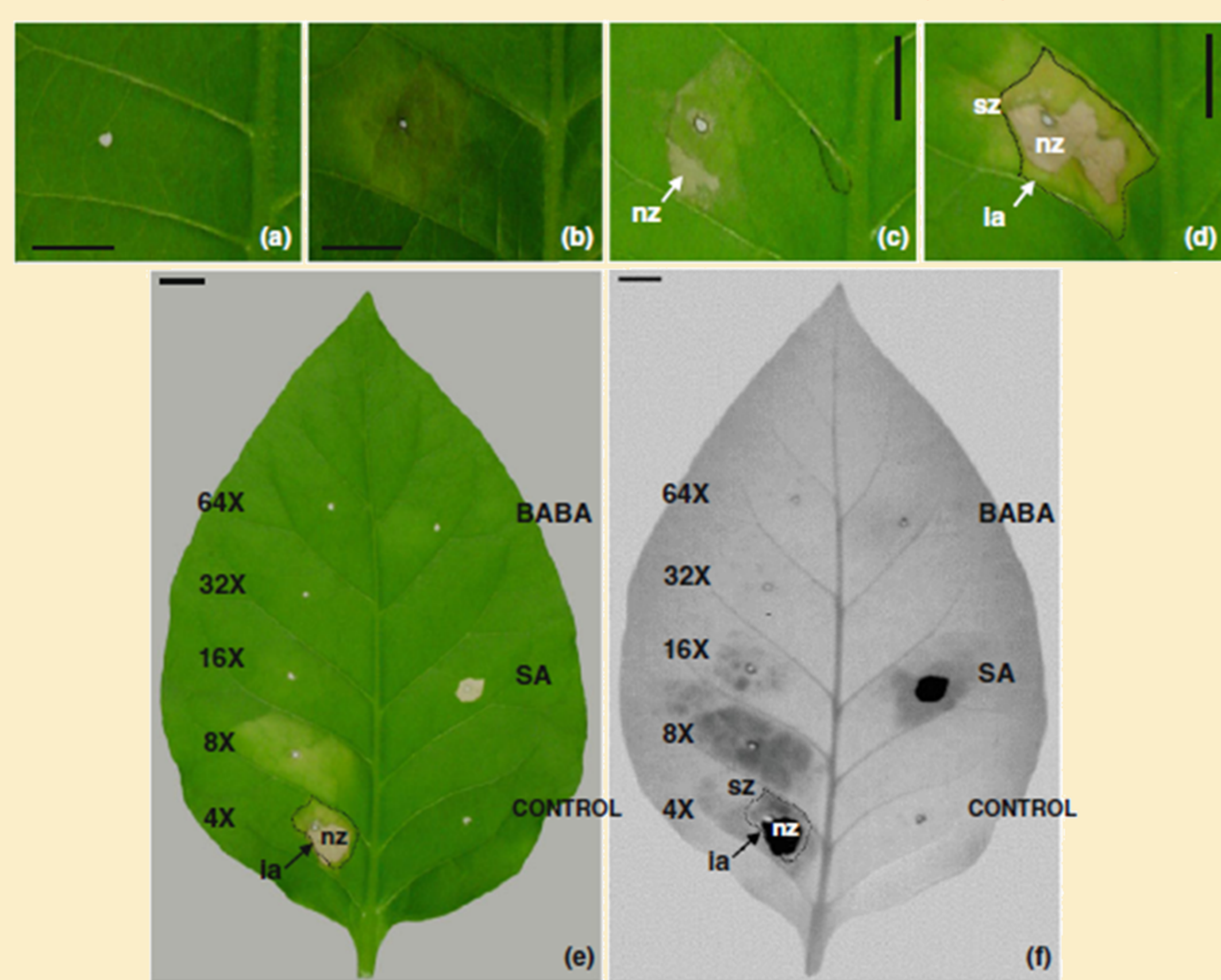
Plant protection has been the major concern in agriculture to improve yields and ensured regular production. Promising molecules have emerged in the 90's with the progress made in plant resistance mechanisms. They are named Plant Defense Inducers (PDIs) ("Stimulateurs de Défenses naturelles") and trigger a molecular and biochemical cascade mechanisms in the host plant to defend against pests. Their mechanism of action is based on plant recognition ability of some elicitor molecules that initiate defense reactions (immunity) *in planta*.

Our group developed a new class of phytosanitary product extracted from grapes having two combining intrant-flux reduction properties i.e. PDIs (patent submitted-DI/CNRS n°3372-01) and pesticide photoprotectors (patent PCT/IB2008/001007). It promotes plant defense and protects chemical pesticides from sunlight prolonging their half life.

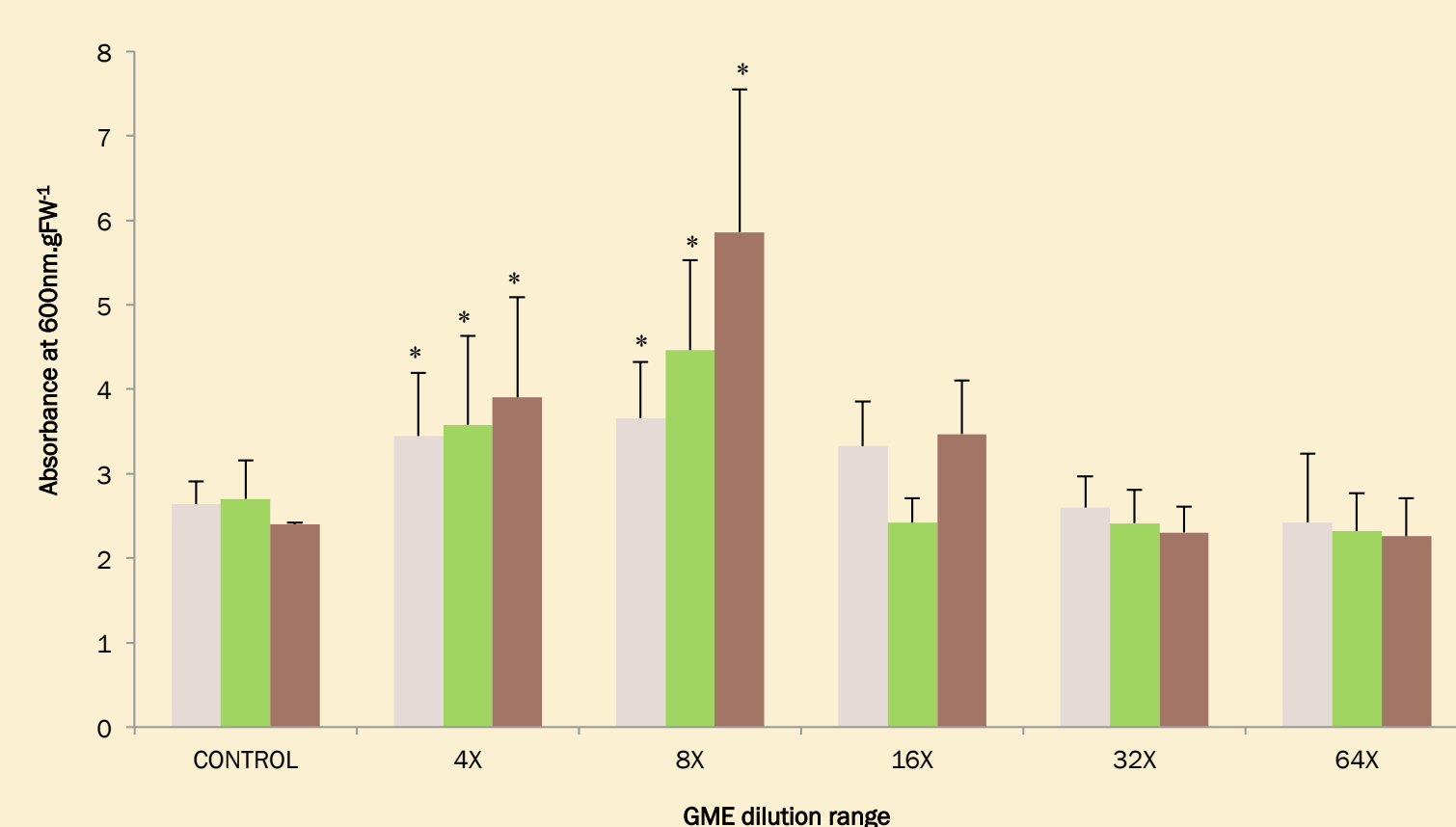
Results

PDI potential

GME mediates local tissue injury



Macroscopic symptoms induced in tobacco (*Nicotiana tabacum* L.) leaves by GME infiltration observed under bright field (a-e) and UV light (f) at 0 dpi (a), 2 dpi (b), 4 dpi (c) and 8 dpi (d-f). GME was infiltrated at 0.25 % (a-d). e, f Tobacco leaves were infiltrated with different GME concentrations: 0.25 % (4x), 0.125 % (8x), 0.0625 % (16x), 0.0312 % (32x), 0.0156 % (64x), ultrapure water (control), 2 mM SA (salicylic acid) and 10 mM BABA. d-f The infiltrated area (ia) is delimited by the black line (dotted line); nz necrotic zone, sz surrounding chlorotic zone. Bars 1 cm



Cell death assayed by Evans Blue staining in tobacco leaves infiltrated with different concentrations of GME. Leaf extracts were prepared from infiltrated tissues at 2, 4 and 8 dpi. The control was infiltrated with ultrapure water. Each independent experiment was performed twice and in triplicate for the same leaf. Bars represent the mean values \pm SE, *P<0.05

GME induced local and systemic PR expression.

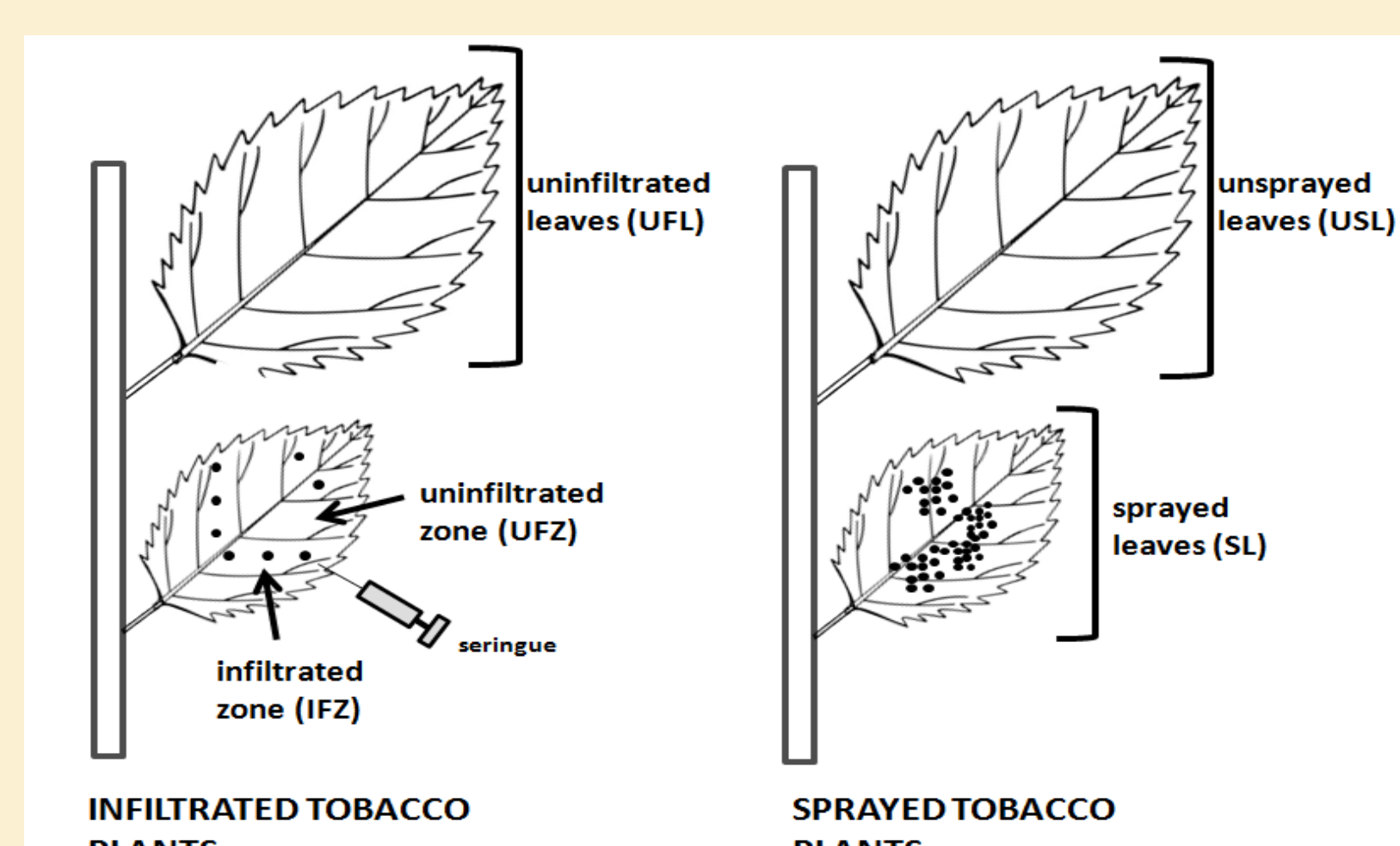
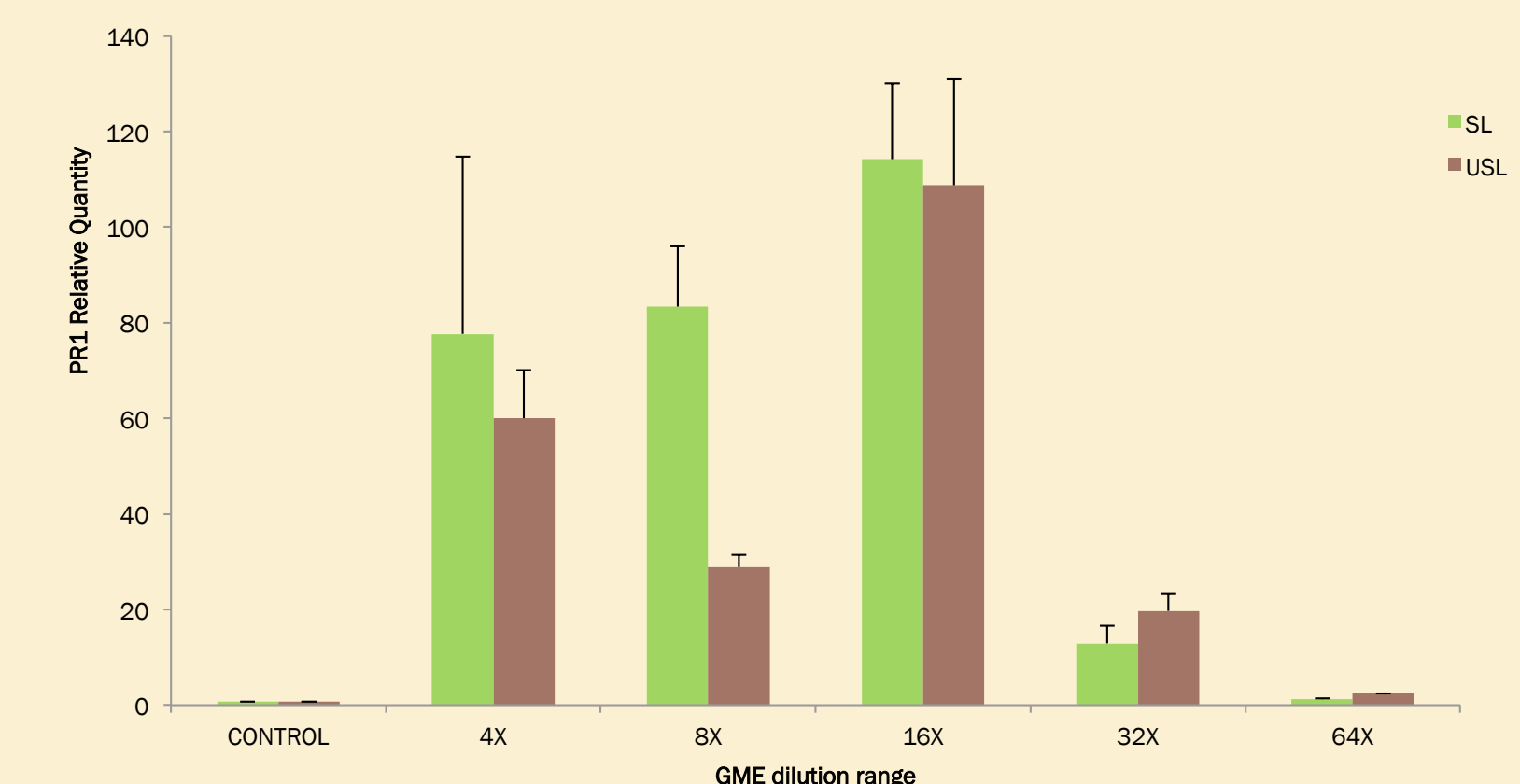
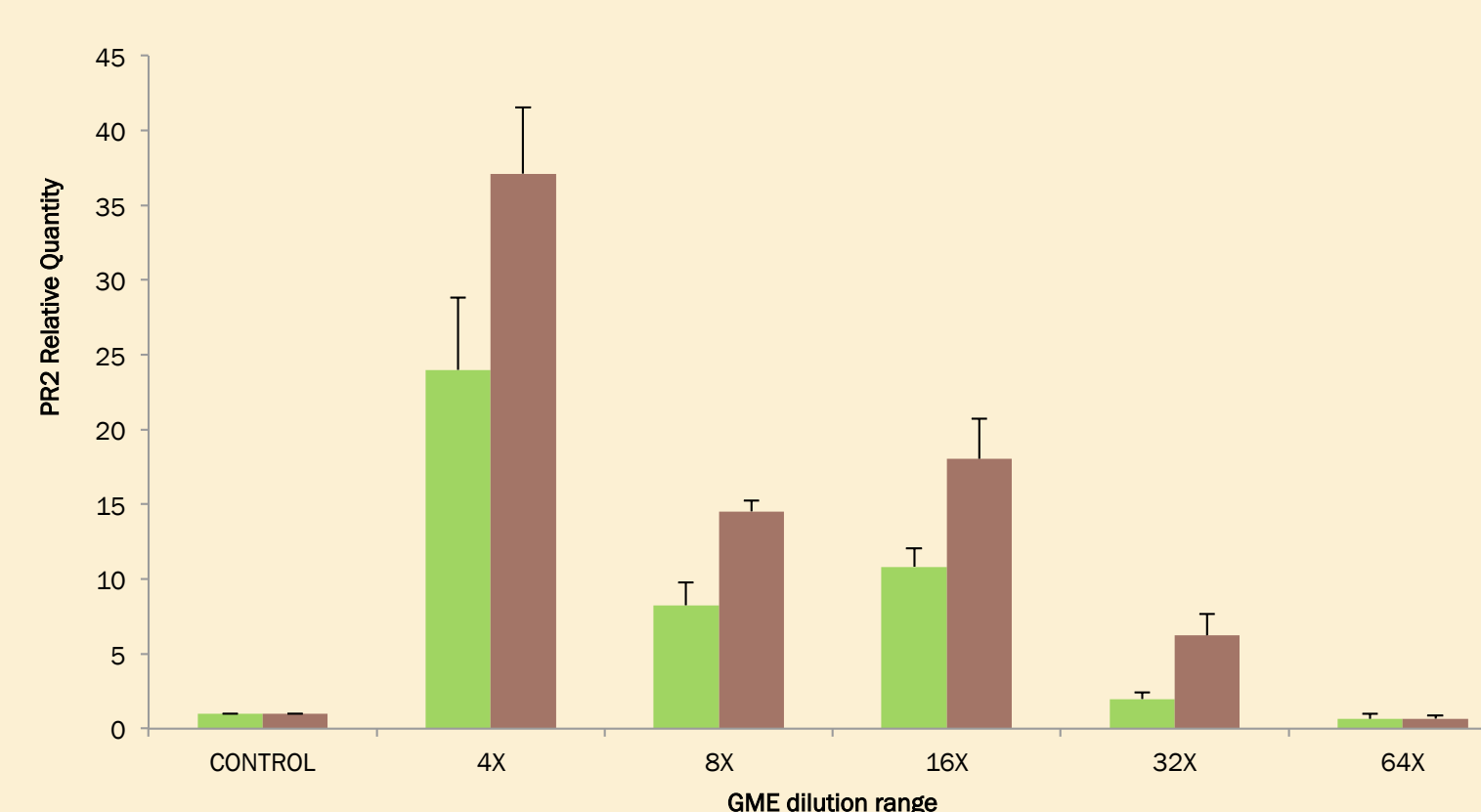
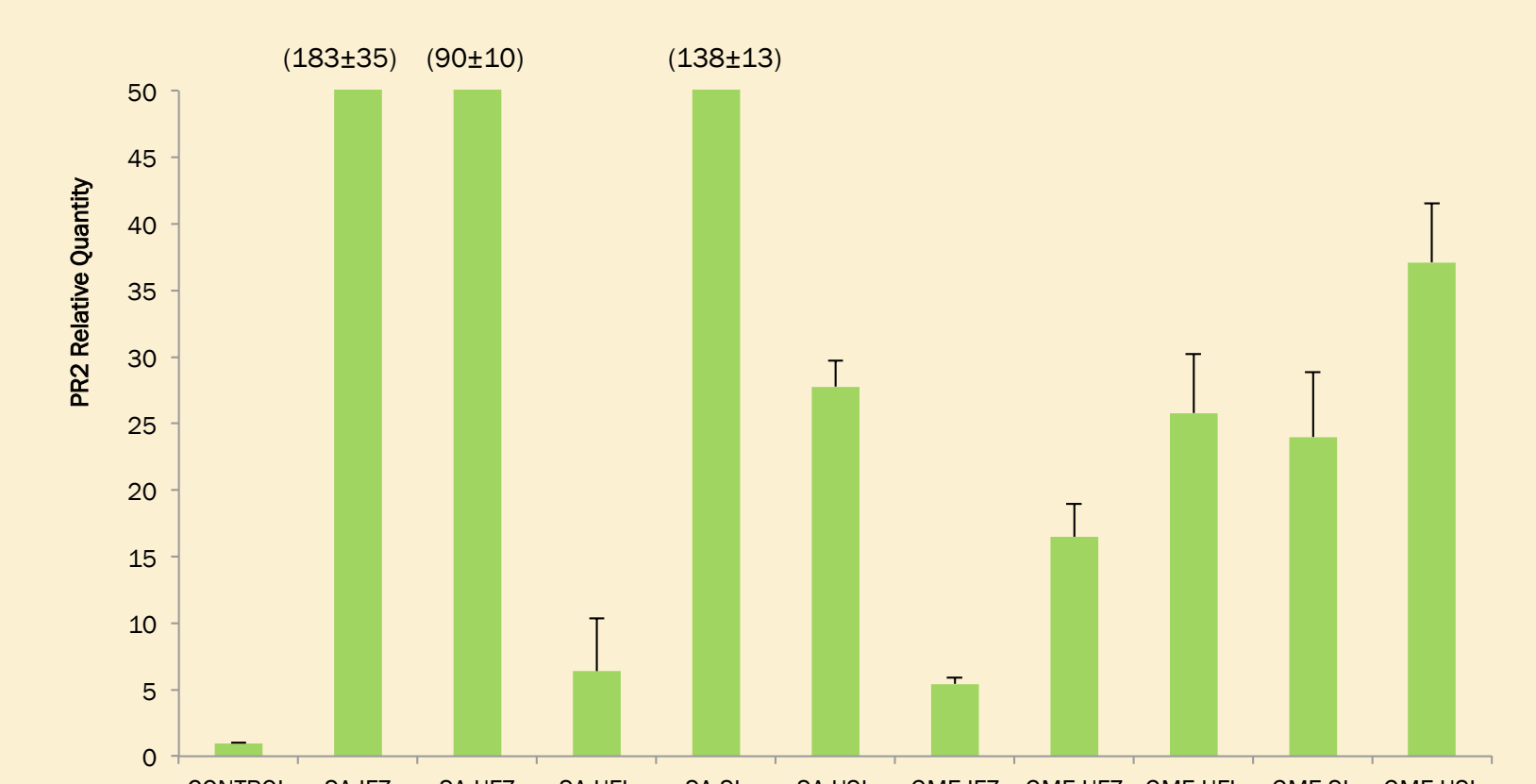
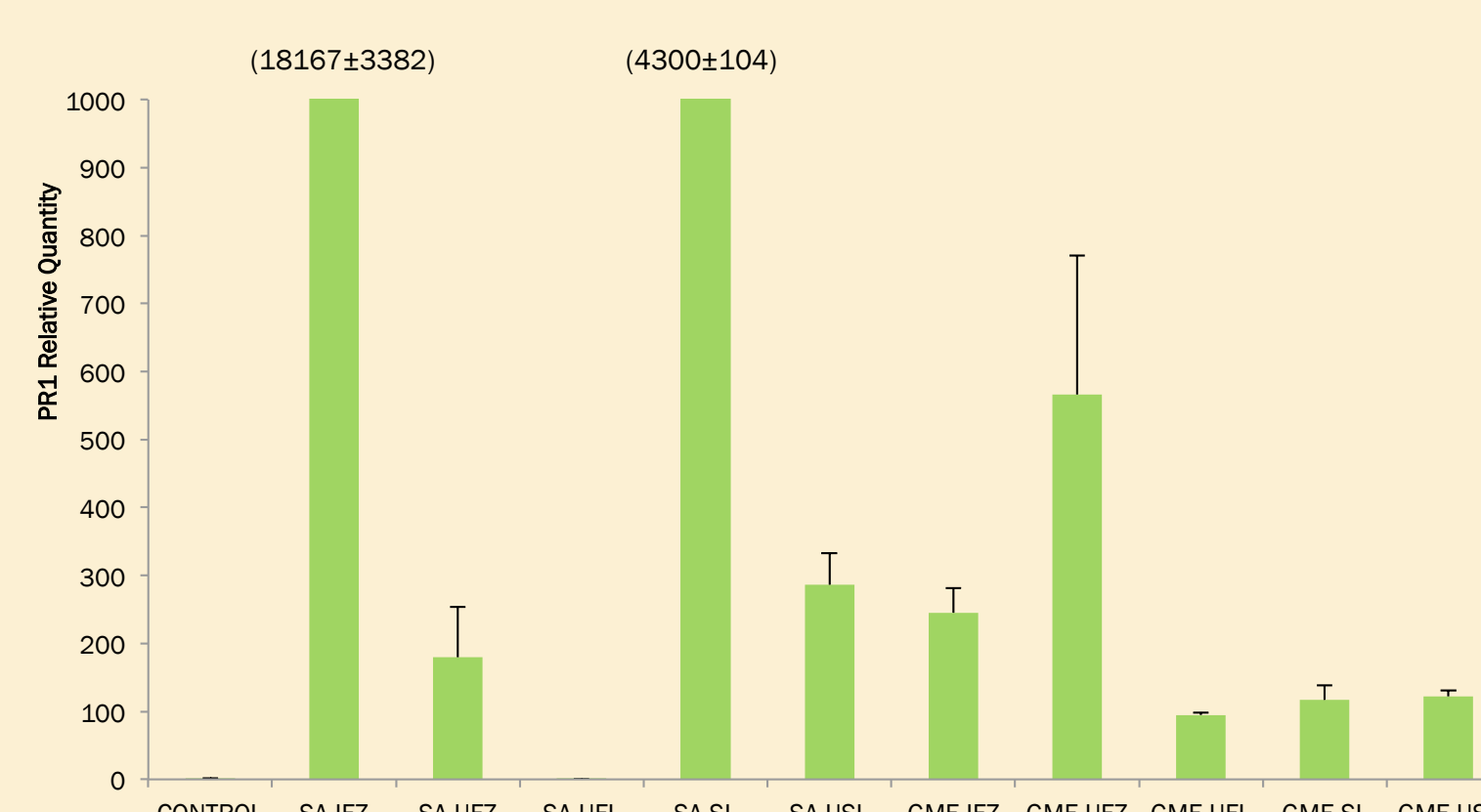


Diagram showing the sampling zones. GME was assayed on 3-month old tobacco plants (preflowering stage). GME was applied as an aqueous solution to foliar tissue either by infiltration or as an aerosol spray. Leaf infiltration was carried out on leaf blades using a plastic syringe. 50 μ l was infiltrated until the solution was spread across a leaf area of 1–2 cm². Foliar spray treatments were administered by spraying onto the adaxial (upper) face of three leaves with a fine atomizer (2 ml per leaf). Leaf tissues were collected from different treated zones.



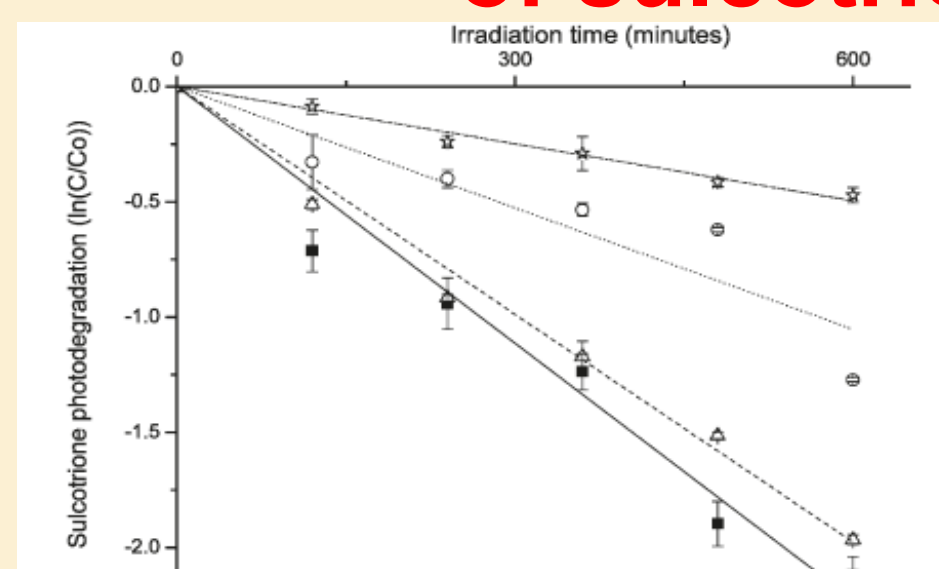
Transcript levels of genes coding for PR (pathogenesis related) proteins after GME treatment were assessed by real time PCR using RNAs isolated from tobacco leaves. The pathogenesis related PR1 and PR2 transcript accumulated both in sprayed (SL) and unsprayed (USL) tobacco leaves at 4 dpi.



PR transcript accumulation in tobacco leaves 4 days after 2 mM SA (positive control) or 0.25 % GME infiltration or spraying. The amount of transcript encoding the PR1 (b) and PR2 (c) genes was quantified by real-time PCR in the IFZ, UFZ, UFL, and after spraying in SL and USL as described above. Values are expressed relative to the control (water treatment) values. Two treated plants were pooled for each experiment, and the results are the means of duplicate experiments. Bars represent the mean values \pm SE

Pesticide photoprotector

Effect of GME on the photolysis of sulcotrione on wax film.

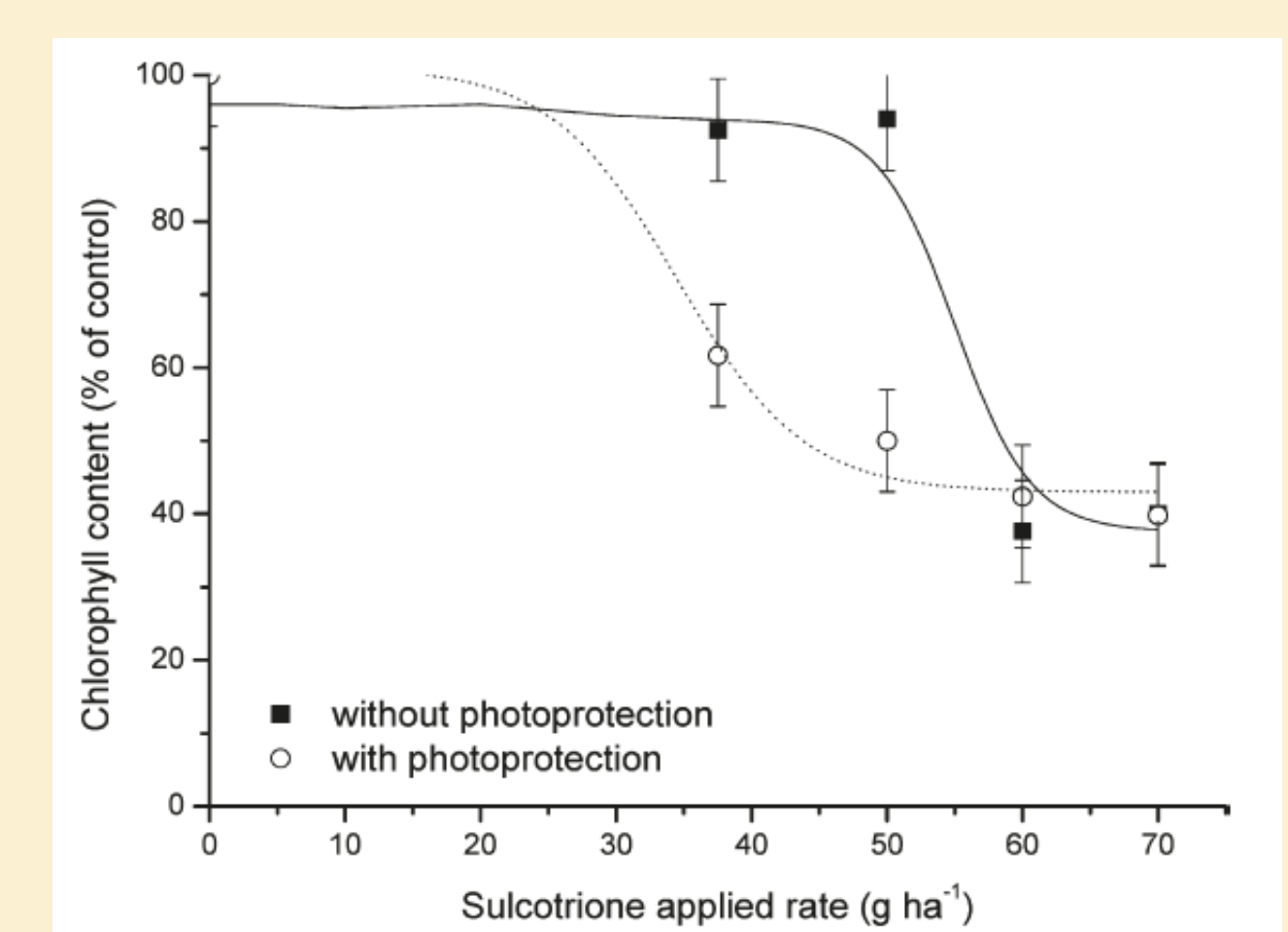


plant extract added	absorbance at 290 nm ²	half-life of photolysis	gain in stability (%)
none		4 h \pm 6 min	
grape wine	0.79	9 h 10 min \pm 5 min	58
sorgium	0.25	6 h \pm 30 min	34
logwood	1.27	9 h 10 min \pm 5 min	58
madder	0.17	5 h \pm 20 min	22
fustic tree	2.82	21 h 20 min \pm 1 h	82
wood	0.06	5 h 40 min \pm 20 min	32
coreopsis	0.39	5 h \pm 20 min	24

Sulcotrione rate of photolysis on carnauba wax at 550 Wm⁻² deposited at a rate of 300 g ha⁻¹ (■) sulcotrione without photoprotection; (△) with madder extract; (O) with coreopsis extract (**) with grape extract. The pigments were added in 3-fold excess compared to sulcotrione.

Effect of photoprotection on herbicidal efficiency.

Grape anthocyanins were added in a 3-fold excess to the sulcotrione solution. Chlorophyll dose response curve 10 days after treatment without photoprotection (dark circles) and with photoprotection (open circles). Data are the results of three replicates.



Conclusions

-The marc extract of red grape cultivars reveals plant defence inducer properties. GME initiate plant defence reactions by local tissue injury induction and PR transcript accumulation.

- The GME-enriched polyphenols and anthocyanins acts as UV-filter and reduces pesticide photodegradation. GME may lead to pesticide reduction use.

- The dual activity of GME (PDI and pesticide photoprotector) makes this natural compound a well-suited product for environmentally oriented crop protection scheme. The uniqueness of this natural product is a result of its origin and nature since it is a high available by-product generated from food industry processing wastes.