Phytopathologia Mediterranea (2013) 52, 2, 289–297

RESEARCH PAPER

In vitro cultures of *Vitis vinifera* L. cv. Chardonnay synthesize the phytoalexin nerolidol upon infection by *Phaeoacremonium parasiticum*

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Summary. This study investigated terpene synthase (TPS) activity and terpene antifungal metabolites in calluses and cell suspension cultures of *Vitis vinifera* cv. Chardonnay infected with *Phaecremonium parasiticum*, one of the fungi associated with the grapevine diseases known as "hoja de malvón" and young vine decline. The highest TPS activity, assessed as tritiated farnesyl pyrophosphate ([$^{1\text{-}3}$H]-FPP) transformed into hexane-soluble radioactive products, was observed in both inoculated calluses and cell suspension cultures (CSC). When tested in inoculated cell suspension cultures the TPS activity was maximal at 8 h after [$^{1\text{-}3}$H]-FPP application and then declined; this was associated with a temporary increase of the sesquiterpene nerolidol. Grape calluses produced: α-pinene, nerolidol and squalene whether or not they were inoculated with *Pm. parasiticum.* As fungal amount raised the relative concentration of α-pinene and nerolidol increased in respect to squalene in calluses. The TPS activity and nerolidol and α-pinene accumulation was correlated with the increase in the amount of inoculated fungus. Of the mentioned metabolites mainly squalene was identified from extracts of fungal cultures. The results suggest that the response of grapevine tissues to *Pm. parasiticum* is dependent on the pathogen concentration and is characterized by increasing TPS activity through *de novo* synthesis.

Key words: grapevine, grapevine trunk diseases, terpene, terpene synthase activity.

Introduction

The grapevine trunk diseases known as "hoja de malvón" (which literally means "geranium like vine leaf") and "young vine decline" have been observed by Argentinean grape growers since the beginning of the last century. These diseases became important in the last two decades when serious damages and important losses of vine yield were found in many wine regions of Argentina (Gatica *et al*., 2000). They are associated with an array of causal agents identified as a fungi complex where *Phaeoacremonium parasiticum*, is one of the most prevalent (Gatica *et al*., 2000 and 2001; Dupont *et al*., 2002; Gatica *et al*., 2004; Lupo *et al*., 2006). *Phaeoacremonium* spp. are plant pathogens localized in vascular tissues that cause stunted growth, wilting and dieback of various woody hosts, especially grapevines. *Pm. parasiticum* was first isolated from

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ISSN (print): 0031-9465
ISSN (online): 1593-2095

diseased grapevine in Mendoza, Argentina (Dupont *et al*., 2002), and subsequently reported in grapevines of Australia, Iran, South Africa and USA (Mostert *et al*., 2006), Chile (Auger *et al*., 2005), Spain (Aroca *et al*., 2006) and Peru (Romero Rivas *et al*., 2009).

Plants have developed different strategies to defend themselves against biotic stress. Among them are the low molecular defensive compounds phytoalexins, produced by secondary plant metabolism (Croteau *et al*., 2000). Knowledge of biosynthesis of phenylpropanoid and terpenoid phytoalexins in different plant tissues provides insights into the mechanisms utilized by plants in regulating these biochemical pathways leading to defense towards pests and diseases (Del Río *et al*., 2001; Amalfitano *et al*., 2009; Degenhardt *et al*., 2009). In this regard, however, there is no information about metabolites effective against *Pm. parasiticum* and little is known about terpenic phytoalexins useful against other xylophagous fungi of woody plants.

It is well known that terpenes are the largest group of plant natural products, comprising at least 30,000 compounds (Croteau *et al*., 2000), with the widest assortment of structural types. Terpenoids form an ample range of structurally related cyclic and acyclic mono-, sesqui-, and diterpenes synthesized by a specific family of enzymes, the terpene synthases (TPS) (Back and Chappell, 1995; Tholl, 2006). These catalysts convert the acyclic prenyl diphosphate and squalene into a multitude of cyclic and acyclic forms, which constitute an essential part of direct and indirect defense systems in ecological interaction against pathogens (Vögelli and Chappell, 1988; Bianchini *et al*., 1999). Studies in several plants show an increase of one type of TPS activity, that of sesquiterpene synthase, and the subsequent accumulation of sesquiterpenes as plant response to pathogen attack (Chappell and Nable, 1987; Choong, 2008). So far there are no effective and environmentally friendly control methods against grapevine trunk diseases, and wine manufacturers' and consumers' negative perception of treatment with synthetic chemicals makes it important to devise alternative methods of control consistent with international regulations controlling organic agriculture (http://www.nal.usda.gov/afsic/pubs/OAP/OAPGuide2.shtml).

On the other hand, attempts to investigate plant defense responses are complicated because the interactions are frequently localized to a few cells and thus the amount of biological material available to the sample is limiting. Also, when investigating an intact host-pathogen system it is often difficult to discern if a response is derived from the plant or from the pathogen. In this way, calluses and cell suspension cultures allow both to analyze cell responses against the pathogen and to compare metabolic changes individually.

Based on above, we hypothesized that grapevine modifies the terpene pathway as defensive responses against *Pm. parasiticum* through production of terpenic phytoalexins. The objectives of this study were to investigate TPS activity and to identify terpene-like antifungal metabolites in *Vitis vinifera* L. cv. Chardonnay calluses and cell suspension cultures exposed to *Pm. parasiticum*.

Materials and methods

Fungal strain

The *Phaeoacremonium parasiticum* used in this work was isolated from wood tissues of diseased grapevines cultivated in Mendoza, Argentina. The fungus was identified according to morphological and molecular characters (Dupont *et al*., 2002) and maintained in glycerol/water solution (10%, v:v), at -20º C in the EEA INTA-Mendoza's fungal collection. For the experiments, *Pm. parasiticum* were maintained on Petri dishes on semi-solid malt extract agar (OXOID Ltd., Basingstoke, Hampshire, England), in a growth chamber at 25 ± 2º C in darkness until the dish surface was partially covered with the fungus. From there, conidial suspensions were prepared in sterile distilled water, and adjusted to 10^7, 10^8 and 10^9 conidia mL^{-1} using a Neubauer counting chamber.

Generation of calluses and cell suspension cultures from *in vitro* plants

Autumn collected vine cuttings (25 cm long) from a vineyard of *Vitis vinifera* L. cv. Chardonnay selected after virus-free indexation were kept in a cold chamber at 5ºC in darkness for 3 weeks. After bud break the cuttings were treated with 100 mg L^{-1} indole-3-butyric acid (IBA, Sigma-Aldrich, St. Louis, MO, USA) for 24–31 h and kept in darkness for root promotion. In order to promote further root and shoot development the cuttings were moved into 2 L pots filled with a mixture of grape compost: sand: vermicompost (7:2:1), disinfected with 500 mg L^{-1} of

streptomycin sulphate, watered weekly with nutrient solution and maintained in a greenhouse with 23 ± 3°C, 40/70 ± 10% RH, and a 16 h photoperiod. After 90 d the plants were dissected in uninodal segments in a laminar flow hood. The explants obtained were surface-sterilized with 70% ethanol 3 min and 0.6% sodium hypochlorite solution (containing Tween-20) for 15 min, and then rinsed 3 times with sterile distilled water. After that, the explants were transferred into glass tubes containing 20 mL of semi-solid Murashige and Skoog culture medium at pH 6.5 (MS; 1962) with 3% sucrose and 0.8% bacteriological agar (OXOID Ltd., Basingstoke, Hampshire, England). The *in vitro* plants were obtained from two consecutive generations of 30 d apical bud explants as follows. Selected apical bud explants were put into glass jars containing 60 mL of half-diluted macronutrients and micronutrients and complete vitamins and iron modified MS medium, plus 10 μg L^{-1} biotin and 5 μg L^{-1} naphthalene acetic acid (NAA, Sigma-Aldrich Inc, St Louis, MO, USA), adjusted to pH 6.5. The culture conditions were maintained at 25 ± 2ºC with 16 h photoperiod of 120 μE m^{-2} s^{-1} provided by white fluorescent lamps. The plants were then used for obtaining calluses and cell suspension cultures.

Apical buds, petioles, and first and second expanded leaves from the *in vitro* plants were collected after 15 d. The leaves were sliced into 3 × 4 mm square pieces, discarding their margins. The explants were placed in glass tubes containing 3 mL of MS medium pH 6.5 with 3% sucrose and 0.8% agar. To improve the calluses quality three combinations of both plant growth regulators, NAA and BA (6-benzyladenine, Sigma-Aldrich Inc, St Louis, MO, USA): 1:1 mg L^{-1}, 5:1 mg L^{-1}, and 2.5:0.5 mg L^{-1}, were assessed. The tubes were sealed with plastic film and maintained in growth chamber at 25 ± 2º C in darkness. After 30 d calluses were evaluated for formation and friability, and the dry weight (DW) was obtained after drying at 60°C until a constant weight was achieved.

Yellowish calluses originated from petioles and subcultured twice (35 d each subculture) in MS medium with NAA and BA 5:1 mg L^{-1} (see above), were transferred to 250 mL flasks containing 70 mL of pH 6.5 MS medium to initiate a set of cell suspension cultures. The cell suspension cultures were maintained and sub-cultured with the MS medium supplemented with NAA and BA, as previously explained, for calluses induction and sub-culture. Then the cultures were incubated in darkness on a rotary shaker at 100 rpm at 25 ± 2ºC to improve quality of the suspensions. The resulting suspension culture consisted of homogeneous single cells and small cell aggregates and therefore were considered of good quality. The viability and the quality of the cell suspensions were monitored by microscopic observations and incubated in semi-solid media in controlled conditions, in order to detect contamination.

Experiments with calluses

Calluses from petioles obtained after two subcultures of 35 d each in MS medium with NAA and BA 5:1 mg L^{-1} (see above) were inoculated with 2 μL of 10^7, 10^8, 10^9 conidia mL^{-1} and 10 μL of 10^9 conidia mL^{-1} suspension of *Pm. parasiticum* and incubated 48 h in a growth chamber at 25 ± 2°C in darkness. As control, a set of three calluses were treated with sterile distilled water without the fungus. After this time each callus was fed with 1 μM of radioactive *trans, trans*-farnesyl pyro-phosphate [$^{1\text{-}3}$H]-FPP (20.5 Ci/mmol, Perkin Elmer, Boston, MA, USA) and 10 μM of farnesyl pyro-phosphate (FPP) triammonium salts, (Sigma-Aldrich, St. Louis, MO, USA) and 72 h later processed for TPS activity and for gas chromatography coupled to electron impact mass spectrometry (GC-EIMS) analysis of metabolites.

Experiments with cell suspension cultures

Cell suspension cultures maintained in MS medium with NAA and BA 5:1 mg L^{-1} (see above) and in the rapid phase of growth (approximately 12 d after subculture) were used in all the experiments. Treatment with the elicitor was initiated by adding 1 mL of 10^6 conidia mL^{-1} of *Pm. parasiticum* into the cell suspension cultures on an orbital shaker (160 rpm) at 25 ± 2ºC in darkness. The controls consisted of sterile distilled water without the fungus and three replicates were performed. After 48 h the cell cultures were fed with 1 μM of [$^{1\text{-}3}$H]-FPP and 1 μM of FPP as a carrier and at intervals of 2, 8, 72 and 96 h the cell cultures were processed for TPS activity and for GC-EIMS analysis of metabolites.

Terpene synthase activity measurements

Calluses and culture suspensions were homogenized in ice-cold mortar with pestle and 800 μL of 1 M potassium phosphate buffer (pH 6.5–7), 20%

(w/v) glycerol, 10 mM sodium metabisulfite, 10 mM ascorbic acid, 15 mM $MgCl_2$, 0.5% PVP (polyvinyl polypyrrolidone, MW 40.000, Sigma Chem Co, St Louis, MO, USA) and 1.47 mM 2-mercapthoethanol. Then, each total protein homogenate was centrifuged for 10 min at 12,000 *g*. After that, the extracts were partitioned into 200 µL of *n*-hexane and treated with 5 mg of silica gel powder (240–300 Mesh, Sigma-Aldrich) to remove any contaminating FPP or farnesol generated by phosphatase activity. An aliquot of 50 µL radioactive *n*-hexane was then poured with 4 mL of Fluka cocktail (Sigma-Aldrich) and radioactivity was measured by using a Tricarb liquid scintillation analyzer (Perkin Elmer, Chicago, IL, USA). TPS activity was expressed as nmol [$^{1\text{-}3}$H]-FPP consumed per mg of protein^{-1}h^{-1} according to Vögeli and Chapell (1988). The protein concentration of the extract was determined by the method of Bradford (Bradford, 1976) using bovine serum albumin (Bio-Rad Laboratories, Philadelphia, PA, USA) as standard. Aliquots of 50–100 µL of the remaining organic phase were used for further GC-EIMS analysis and product identification.

Terpene determination

The hexane phase from calluses and cell suspensions was analyzed by GC-EIMS in order to identify terpene-like products and to estimate their relative concentrations. Two µL of each sample were injected in split-splitless mode in a GC-EIMS system (Clarus 500, Perkin Elmer). The GC column was a Perkin-Elmer Elite-5MS, cross-linked methyl silicone capillary column (0.25 mm internal diameter, 30 m long and 0.25 µm film thickness) eluted with He (0.7 mL min^{-1}). The oven temperature program was 45ºC for 1 min, followed by an increase of 2ºC min^{-1} to 130º C, 20º C min^{-1} to 250º C and held at 250º C for 10 min. The ionization potential was 70 eV and a range of 30 to 400 atomic mass units was scanned. Terpene products from the assays were identified according to Gil *et al*. 2012; by comparison of retention times and full scan mass spectra with a set of authentic standards, nerolidol, pinene, and squalene (Sigma-Aldrich Steinheim, Switzerland) and by comparison with those standards of the National Institute of Standards Technology (NIST) library. To estimate the concentration of the different metabolites 50 ng µL^{-1} of *n*-hexadecane (Supelco, Bellefonte, PA, USA) as an internal standard was added to each sample.

Antifungal activity bioassays

For evaluation of antifungal activity, 4 discs of filter paper (0.5 cm in diameter) were impregnated with 5 µL each of pure authentic nerolidol (97% Sigma-Aldrich, Steinheim, Switzerland) and were equidistantly distributed on semi-solid malt extract agar dishes with *Pm. parasiticum* grown for 8 days. All cultures were incubated at 25 ± 2° C until the mycelium of fungi reached the edges of the control prepared without nerolidol, and the antifungal index was calculated (Bittner *et al*., 2009; Park *et al*., 2009). Each treatment had 5 replications. The antifungal index was calculated as follows:

Antifungal index (%) = (1 - Ds / Dc) × 100; where, Ds is the diameter of the hyphal growth in the Petri dish treated with nerolidol, and Dc is that of the control.

Statistical analysis

The normal data were performed by analysis of variance (ANOVA) and multiple range tests at 95% confidence level. The software Statgraphics Centurion XV version 15.0.10 (Stat point Technologies Inc., Warrenton, VA, USA) was used.

Results

Generation of calluses from *in vitro* plants

The calluses formed in most of the different conditions from 57 to 100%, except for the first leaf callus with the combination of NAA-BA 1:1 mg L^{-1} where calluses formation decay to 28%. The calluses friability was low in the NAA-BA 1:1 mg L^{-1} combination, and the treatments with NAA-BA 5:1 mg L^{-1} showed the best calluses development, greater friability and higher DW. Regarding the explant selection it was observed that calluses from petioles and apical buds had the highest friability and DW (Table 1).

Terpene synthase activity measurements on calluses and cell suspension cultures

Increases of TPS activity was found in calluses inoculated with the highest conidial suspension of *Pm. parasiticum* (Figure 1). The major TPS activity analyzed as [$^{1\text{-}3}$H]-FPP consumed (nmoles mg protein^{-1} h^{-1}) was found in the hexane-soluble fraction of calluses inoculated with 2 µL and 10 µL of 10^9 conidia mL^{-1}

Table 1. Calluses formation from apical buds, first and second leaf, and petiole, in three combinations of plant regulator concentrations, NAA-BA 1:1, NAA-BA 5:1 and NAA-BA 2.5:0.5 mg L^{-1}.

Concentration [a]	Origin	Callus		
		Formation (%)	DW [b] (mg)	Friability (%)
1:1	Apical bud	71	16.5	40
	First leaf	28	4.5	50
	Second leaf	86	10.5	33
	Petiole	71	29.4	100
5:1	Apical bud	71	37.2	100
	First leaf	86	15.6	33
	Second leaf	100	13.6	86
	Petiole	100	32.1	100
2.5:0.5	Apical bud	57	36.9	100
	First leaf	86	12.0	33
	Second leaf	86	29.0	83
	Petiole	100	41.4	86

[a] Plant regulator concentrations, NAA-BA 1:1, NAA-BA 5:1 and NAA-BA 2.5:0.5 mg L^{-1}.
[b] DW, callus dry weight.

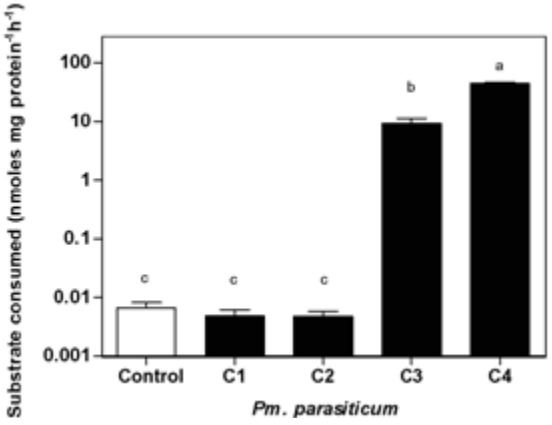

Figure 1. TPS activity expressed as nmoles of [$^{1\text{-}3}$H]-FPP consumed per mg protein^{-1} h^{-1} in calluses inoculated with 2 μL 10^7 (C1), 10^8 (C2) and 10^9 (C3) or 10 μL 10^9 (C4) conidia mL^{-1} and in control.

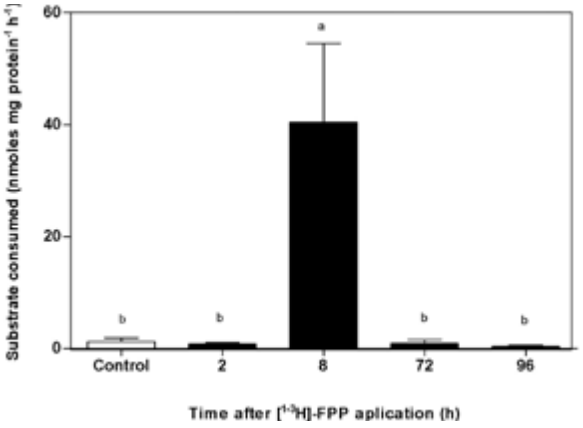

Figure 2. TPS activity expressed as nmoles of [$^{1\text{-}3}$H]-FPP consumed per mg protein^{-1} h^{-1} of cell suspension cultures at 2, 8, 72 and 96 h post application of FPP radioactive, and previously inoculated with 1 mL of 10^6 conidia mL^{-1} of *Pm. parasiticum*.

(C3 and C4, respectively), with statistically significant differences with respect to lower concentrations 2 μL of 10^7 and 10^8 conidia mL^{-1} (C1 and C2, respectively) and the controls without the pathogen ($P \leq 0.00001$).

Figure 2 shows the results of the time-course experiments with cell suspension cultures incubated 48 h with *Pm. parasiticum* as elicitor and the control

without the elicitor, followed by the addition of the radioactive FPP. TPS activity, measured at intervals of 2, 8, 72 and 96 h after [$^{1\text{-}3}$H]-FPP application, was maximal at 8 h post radioactive FPP application and then declined progressively at 72 and 96 h showing not statistically different between the TPS activity from cell suspension cultures incubated with *Pm. parasiticum* as elicitor and the control without the elicitor. This remarkable increase in activity in cell suspension cultures infected with the pathogen in a specific time, strongly suggest *de novo* synthesis of TPS ($P = 0.0018$).

Terpenes determinations on calluses and cell suspension cultures

When the hexane control fractions of grape calluses, were analyzed by GC-EIMS, the most important compound identified was the triterpene squalene (Figure 3A, s). Meanwhile the sesquiterpene nerolidol, the monoterpene α-pinene and also the triterpene squalene (Figure 4A, n, p and s, respectively) were observed in calluses inoculated with the fungus. Moreover, along with the increases in fungal amount it was observed an increase in the relative concentration of α-pinene (from 30 to 383 ng mg^{-1} FW) and nerolidol (from 70 to 915 ng mg^{-1} FW) respect to squalene (from 10 to 49 ng mg^{-1} FW). The highest relative concentration of nerolidol and α-pinene was found in calluses inoculated with 10 µL of 10^9 conidia mL^{-1} (Table 2), while in the control without the pathogen the terpene squalene prevailed (Table 2). Also, was notable the amount of nerolidol in the inoculated cell suspension cultures 8 h post-application of radioactive FPP, was far and away the most abundant compound found, with an estimated amount of 1800 ng mg^{-1} DW (Figure 5A, n).

When the fungal culture was controlled throughout the growth phase and the TPS and terpene metabolites were analyzed, neither significant TPS activity nor terpenes presumably involved in plant defense were found, except for squalene (13 ng mg^{-1} FW).

Antifungal activity bioassays

The mycelial growth of *Pm. parasiticum*, was inhibited at 54% by pure nerolidol at a concentration

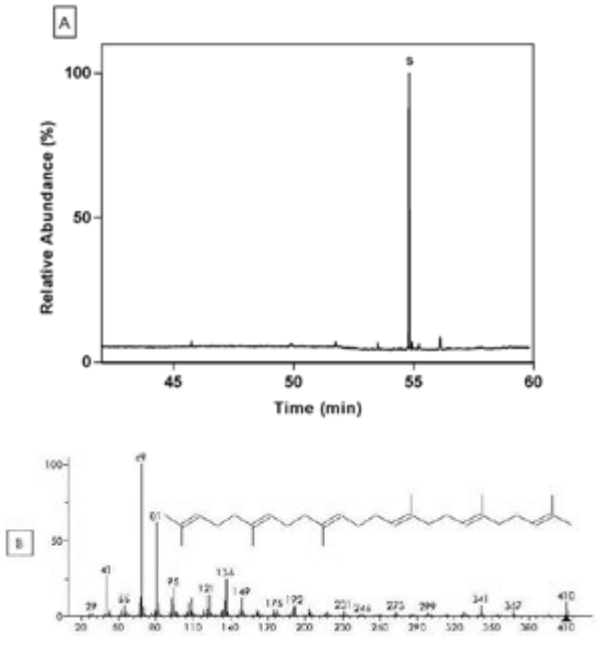

Figure 3. (A) Total Ion Chromatogram (TIC) from extracts of calluses without elicitor. The peak at 54.81 min (s) corresponds to squalene. (B) MS spectra of squalene.

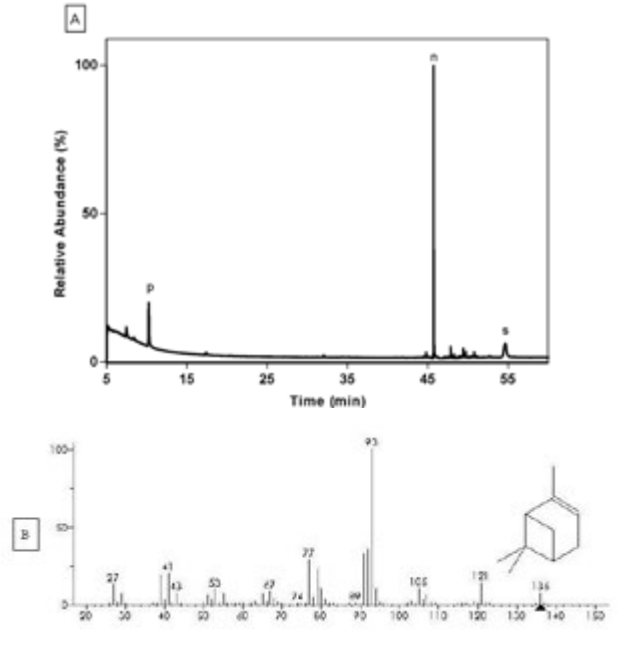

Figure 4. (A) TIC from calluses inoculated with 10 µL of 10^9 conidia mL^{-1}. The peaks in TIC correspond to α-pinene (10.28 min, p), nerolidol (45.74 min, n), and squalene (54.65 min, s). (B) MS spectra of α-pinene.

Table 2. Relative quantities of squalene, α-pinene and nerolidol calculated respect to a known amount of *n*-hexadecane (ng mg^{-1}) by GC-MS, in samples of calluses non inoculated (control) and inoculated with different concentrations of *Pm. parasiticum*.

Compounds	Control [a]	*Pm. parasiticum* [b]			
		C1	C2	C3	C4
Squalene	694	10	10	37	49
α-pinene	27	30	96	211	383
Nerolidol	51	70	255	478	915

Squalene, α-pinene and nerolidol expressed in ng mg^{-1}, calculated as the relationship among the total ion chromatographic peak area respect to a known amount of *n*-hexadecane in:
[a] Calluses non inoculated (control).
[b] Inoculated with 2 μL of 10^7 (C1), 10^8 (C2) and 10^9 (C3), and 10 μL of 10^9 (C4) conidia mL^{-1} of *Pm. parasiticum*.

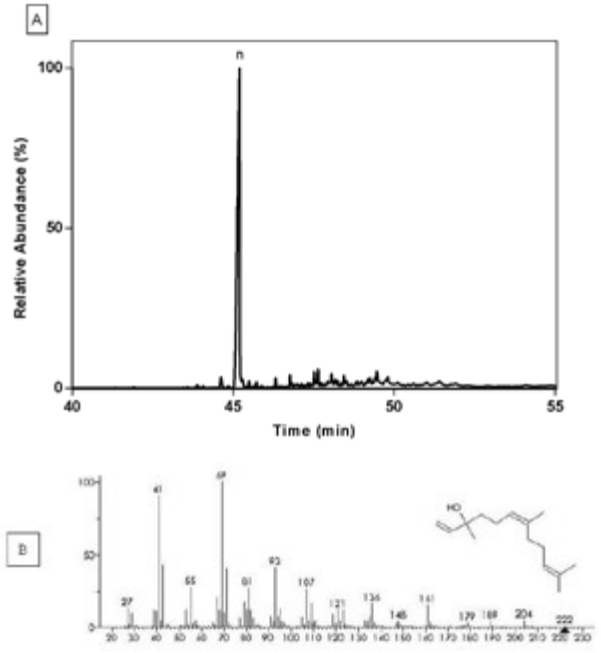

Figure 5. (A) TIC of cell suspension cultures inoculated with *Pm. parasiticum* and analyzed 8 h after application of [$^{1-3}$H]-FPP; the peak at 45.20 min was identified as nerolidol (n). (B) MS spectra of nerolidol.

of 4.2048 μg $disc^{-1}$ (Figure 6). Even though studies have been made of this sesquiterpene in other interactions, this is the first report of antifungal activity of nerolidol in a grapevine pathogen.

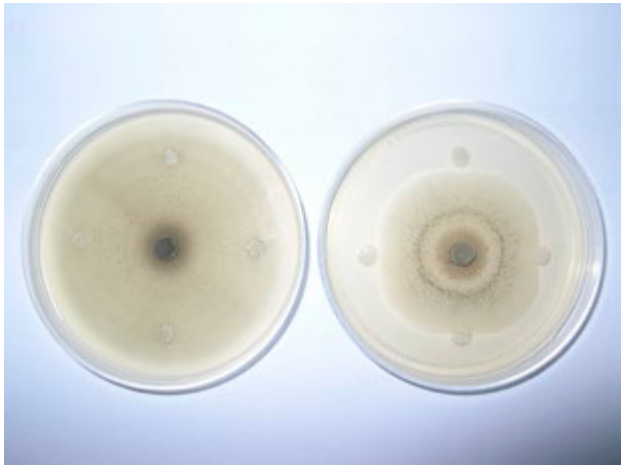

Figure 6. Effect of nerolidol on *Pm. parasiticum* growth in a Petri dish with 15 mL of malt extract agar (right) and *Pm. parasiticum* growth without nerolidol (left).

Discussion

The results from this study support the proposed hypothesis, that is, calluses and cell suspension cultures respond to the elicitor *Pm. parasiticum* by increasing TPS activity and synthesizing specific terpenoids compounds.

The experiment with inoculated calluses showed that the fungal concentration is important in eliciting defensive responses by increasing TPS activity (according to the conidial dilution). This agrees with other reports in which it was observed that the accumulation of capsidiol was proportional to the amount of elicitor added (Chappell *et al*., 1987). Also, by studying the activity of TPS over time with radio-labeled substrate on infected cell suspension cultures, it was found that 2 h after feeding with radio-labeled FPP substrate the TPS activity was scarce, while high activity was registered after 8 h and then declined again, suggesting that the cells respond against the pathogen through *de novo* TPS synthesis. In the studied calluses and cell suspension cultures inoculated with the pathogen, there was a clear correlation between TPS activity and a certain types of terpenes presumably involved in plant defense. Dudley (1986) and Croteau *et al*. (1987) have suggested the accumulation of mono-, sesqui-, and diterpenes in plant tissues challenged by pathogens correlated with an induction of the respective cyclase-like enzyme activities. Also Kapper

et al. (2005) showed that transgenic plants constitutively emit compounds of the complex herbivore-induced volatiles from the sesquiterpene nerolidol, the first intermediate in the route to nonatriene. Our experiments show the presence of nerolidol in calluses and in cell cultures inoculated with the pathogen, while α-pinene was identified in minor amount in calluses only. Regardless of these differences observed amongst the biological systems used, the induction of the TPS activity and the subsequent increase in terpenes against fungus attack were transient since it was found in a given time in the CSC. This is the first report of these compounds being associated with grape-pathogen interaction, although they have been related as phytoalexins in other plant species. In fact, significant increases in the levels of the monoterpenic olefins α-pinene, β-γ-pinene, δ-carene, and δ-phellandrene, which are thought to be toxic to fungus, were found in stem tissue of *Pinus contorta* infected with *C. clavigera* (Croteau *et al*., 1987). As well, Bayonove (1989) observed that *Botrytis cinerea* would affect the biosynthesis of monoterpenes in different grape varieties.

The results obtained with calluses and cell suspension cultures on different experiments, indicate that the terpene compounds characterized by GC-EIMS after the host-pathogen interaction are exclusively synthesized by the plant tissues as defensive mechanisms to the pathogen attack. That is, the vine would be responding to the pathogen attack altering the pattern of terpene biosynthesis by inducing the synthesis of specific mono- and sesquiterpenes. In parallel, a drastic decrease of squalene concentration, key triterpene in the synthesis of sterols, was found, re-enforcing the idea of remarkable changes in the secondary metabolism of infected plants.

We have observed the effect of nerolidol on *Pm. parasiticum* grown in agar medium, and it is noticeable that the standard nerolidol retard the fungal growth by 54%. Therefore, the antifungal activity of terpenes like citral, eugenol, nerolidol and α-terpineol against *Trichophyton mentagrophytes* may act by affecting its normal metabolism (Park *et al*., 2009).

In conclusion, in our study we found that nerolidol possess antifungal activity that inhibits the growth of *Pm. parasiticum.* Due to its antifungal property, and considering that the USA's Environmental Protection Agency qualifies nerolidol as an innocuous natural volatile compound, its use may be advisable for the treatment of "hoja de malvón" and "young vine decline" diseases as an organic, environmentally-friendly antifungal ingredient.

Acknowledgements

The authors thank the technical assistance of L. Bolcato in GC-EIMS determinations, M.T. Ponce in *in vitro* cultures, and S. D´Innocenzo in pathological assays. This work was supported by funds from Instituto Nacional de Tecnología Agropecuaria (INTA) (AEPV-2551) to M. Gatica, Fondo para la Ciencia y Técnica (Foncyt) PICT 20-20093 to R. Bottini, Consejo Nacional de Ciencia y Técnica (CONICET) PIP 5208 to R. Bottini, and PAE-PICT-2007-02190 to P. Piccoli.

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Accepted for publication: September 14, 2012