Electronic Journal of Biotechnology ISSN: 0717-3458 Vol.9 No.3, Special Issue, 2006

DOI: 10.2225/vol9-issue3-fulltext-4 RESEARCH ARTICLE

Morphological and chemical diversity in the Type IV glandular trichomes of Solananeae (*S. sisymbrifolium* and *N. glauca*) as germplasm resources for agricultural and food uses

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Financial support: CSIC Nº203 and CONICYT Nº122.

Keywords: antifungic activity, interespecies variability, mycotoxins, sugar esters.

Abbreviations: CC: column chromatography
GC-MS-EI: gas chromatography - mass spectrometry - electronic impact
MALDI-TOF: Matrix Assisted Laser Desorption Ionization-Time of Flight
Ng: *Nicotiana glauca*
NMR: Nuclear Magnetic Resonance
SE: sugar esters
SEM: Scanning Electron Microscopy
Ss: *Solanum sisymbrifolium*

Morphological variation in type IV trichomes in *Ss* and *Ng* was studied through SEM. The differences can be related to chemical differences in the excreted sugar esters. *Ng* trichomes exude two fractions, one of glucose

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tri-esters and the other one of sucrose tetra-esters, in a 3:7 ratio. The main acid found forming these esthers, is 3-methylvalerianic acid, in consonance to those secreted by other Solanaceae. Esters from *Ss* are novel structures, which can also be separated into three fractions, two of arabinoxylans, and the other one of arabinose, all glycosilated with β-hydroxipalmitic acid and sterified with the C_{12}-C_{16} acids. All five fractions have antifungic activity at µg/cm^2 concentrations, both against common and mycotoxigenic fungi, such as *A. niger, A. flavus, P. chrysogenum* y *P. expansum*. *A. flavus* does not grow in the presence of the SE of *Ss* and is not insensible to those from *Ng*, but these last are more effective in the inhibition of *P. expansum*, the other mycotoxigenic fungi studied. The differential antifungal activity observed gives the plant protection against a wide spectrum of fungi, resulting in a better adaptation to the environment. Both plants are common weeds, with the potential of contributing to germplams lines in the improvement programmes of crops such as *L. esculentum,* and their extracts can be used as natural fungicides to protect crops and plantations.

70% of all Solanaceae are pubescent, and the detailed study of the leaf ultrastructure shows the presence of hairs and glandular trichomes, structures that have a well established defensive role in the interaction of plants and their ecosystems (Goffreda et al. 1989; Juvik et al. 1994; Harborne, 2001). The chemical secretions of Type IV peduncular trichomes are composed by complex mixtures of esters of short chain fatty acids and simple sugars such as glucose and sucrose (King et al. 1990; Steffens, 2000; van der Hoeven and Steffens, 2000). The conventional improvement programmes for tomato and potato have been aimed at the selection of vegetal materials where these trichomes were present, as they give the plants natural resistance to pests (Goffreda et al. 1989; Shapiro et al. 1994). In a systematic screening to identify new native Solanaceae germplasm sources with sugar esters (SE), we have described the insecticidal properties of *Ss* and *Ng* extracts (Cesio et al. 2000). The present work is the continuation of the studies on the chemical and biological properties of their exhudates, that represent results of possible agricultural interest.

Table 1. Fungal inhibition by SEs.

	Solanum sisymbrifolium			*Nicotiana glauca*	
	M1[a]	M2[a]	M3[a]	ES	EG
A. niger	42	48	36	19	28
A. flavus	--	56	38	--	--
P.chrysogenum	42	--	34	24	34
P. expansum.	25	19	15	5	7

[a]inhibition halo in µg/cm^2

MATERIALS AND METHODS

Isolation of trichomes secretions

In large quantities. The aerial parts, which contain the trichomes, are submerged 30" in acetonitrile. The solvent is removed by rotaevaporation and the residue is fractioned by CC.

Table 2. Activity of common food preservatives and SEs from *Ng*. Results in µg/cm^2.

Compound/fraction	*A. Niger*	*P. chrysogenum*
Sucrose esters	19	24
Glucose esters	28	34
Propylparaben	100	75
Ortophenylphenol	10	18

Exclusively from the trichomes. 1) The aerial parts, frozen in liquid nitrogen, are mixed with ground dry ice and shaken in a vortex mixer at high speeds. The vegetal material is removed and the dry ice is allowed to sublime. The isolated trichomes are extracted with acetonitrile. 2) The contents of the heads of the trichomes is extracted with a microsyringe using a magnifying glass.

Structural elucidation. The extracts are fractionated by CC using a $CHCl_3$:MeOH 100:0-90:10 gradient. The SE fractions are studied by conventional procedures to establish their sugar and fatty acid contents. The pure compounds are studied by spectroscopical methods to establish their structures [NMR (400MHz); MALDI-TOF-MS; GC-EI-MS].

Antifungal activity. A quantitative bioautographic method was used (García et al. 1997).

SEMs were as gold replicas.

RESULTS AND DISCUSSION

The peduncular trichomes of the two species are different (Figure 1). The variability is observed in their morphology, in their location in the plants and in the chemical compositions of their secretions, which present different antifungal activities.

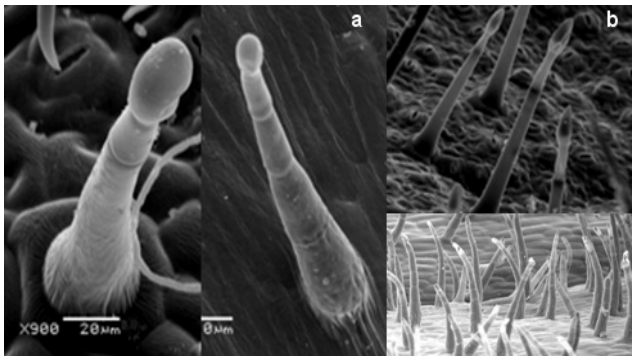

Figure 1. Trychomes Type IV.
(a) *S. Sisymbrifolium* trychome.
(b) right, *N. Glauca* flower trychome.

Trichome location and morphology

Ng trichomes are only present in the inflorescences. Morphologically they have a long pluricellular (n > 5) peduncle and can break from their base. They have also globular heads that grow turgid as they fill with exudate. *Ss* trichomes are on both faces of the leaves, being denser in the abaxial side. They have fewer cells (n < 4) in their peduncles, do not break off at the base, and have oval multilayered heads.

Exudates chemical composition

The exudates were selectively extracted to verify their origin in the trichomes under study. It this way it could be established that the SE isolated from the trichomes and present in the epicuticular waxes were identical for each species. There is an interspecific variation in there compositions, as *Ng* excretes esters of sucrose and glucose in a 7:3 ratio with 3-methylvalerianic acid as main component (78%). Figure 2 shows the complexity of these fractions, which could be resolved by careful analysis of the NMR and MS spectra, and comparison with bibliographic data (Severson et al. 1994; Ohya et al. 1996). The main component, which is representative of the whole fraction, is sucrose -2,3,4 tri(3-methylvalerianate) -1'monoacetate. The other constituents of the fraction have other acids linked to the sugar. In the case of the glucose esters the main component is the trivalerianate.

The esters of *Ss* are novel structures, composed by arabinose and arabinoxilanes glicosilated with β-

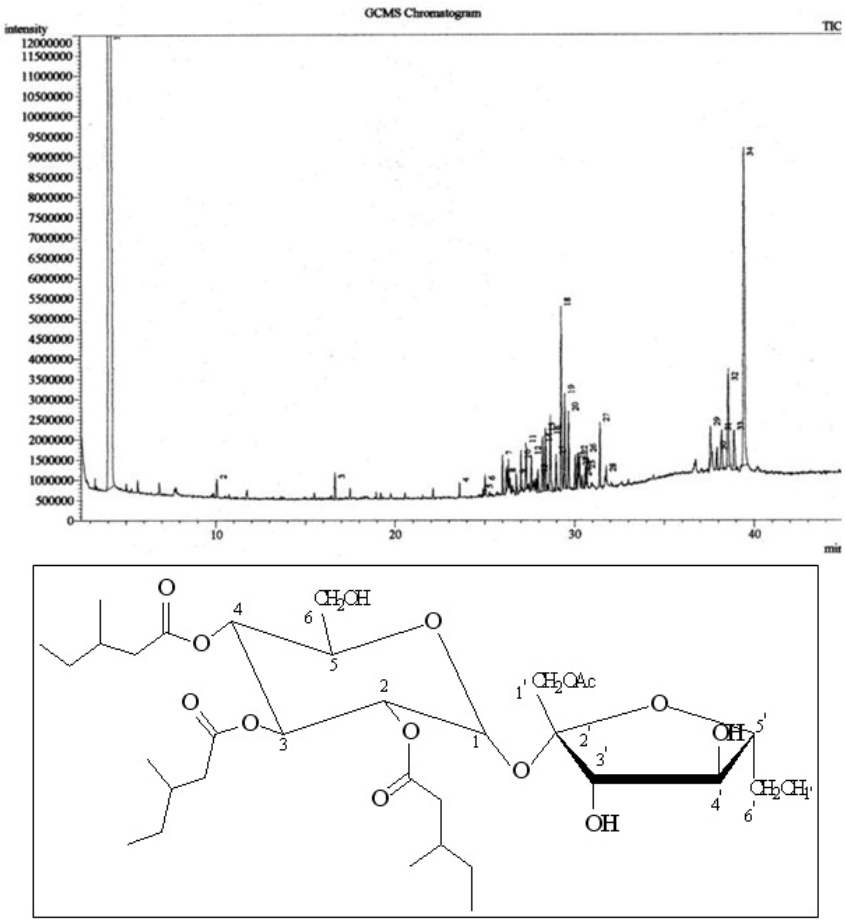

Figure 2. GC-MS of the sugar TMSi esters of *N. glauca.*

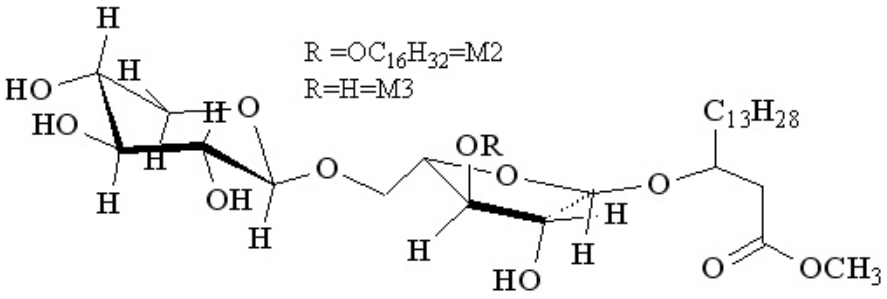

Figure 3. Structures of *S. sisymbrifolium* M2 y M3.

hydroxipalmitic acid, esterified with fatty acids such as lauric, miristic and palmitic. Three groups of components can be separated by CC, M1, M2 and M3 (5%, 28% y 67%). By NMR COSY, HMQC and HSQC-TOCSY bidimensional experiments, M2 could be established to be β-xylopyranosil (1-5)-α-furoarabinose esterified with palmitic acid and glycosilated with one unit of β-hydroxipalmitic acid. In the case of M3 is the non-esterified glycoside (Figure 3). The structure of M1 corresponds to a arabinose polyesterified with lauric, miristic and palmitic acids, and glycosilated with β-hydroxipalmitic acid. The molecular weight was established by MALDI, and the sugars were analysed by the conventional alditol technique.

Antifungal tests

In most cases phytopathogenic fungi have to penetrate the epicuticular wax layers before invading a plant (Kolattukudy, 1995). Due to this it is common to find antifungal compounds in the external surface of plants (Kennedy et al. 1992). The traditional agar diffusion assay to evaluate antifungal compounds is not applicable for lypophilic compounds (García et al. 1997). Such substances do not diffuse in agar, a circumstance that can result in false negatives. Prior work from our group with a modification of Rahalison's technique (Rahalison et al. 1991; Larramendi et al. 1998) has shown that Bioautography on TLC is particularly useful to assay bioactive compounds present in complex mixtures, as shown in Figure 4, with a quantitative perspective.

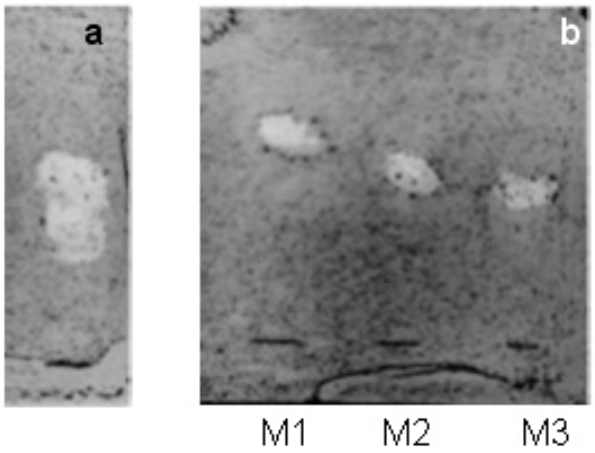

Figure 4. Bioautography using *A. niger* of the SE fractions of *Ng* (a) and *Ss* (b).

The results can be presented in mass/area units, with valuable information on the bioactivity of the substance(s) under study. The antifungal assays are shown in Table 1.

The fractions show differential activity to the assayed fungi, linked to the different chemical structures involved. This is particularly interesting in the case of fungi that contaminate foods and are mycotoxigenic, as *A. flavus* and *P. Expansum* (Pitt and Hocking, 1999). *A. niger* is inhibited by all fractions, although those from *Ng* are most active. *P. chrysogenum* is inhibited by *Ng* esters and by the M3 and M1 fractions of *Ss*. The *Ng* fractions are the most active. The two arabinoxilanes from *Ss,* M2 y M3, inhibit aflatoxin producing *A. flavus.* This is the first report of inhibition of *A. flavus* by SE. *A. niger* and *P. expansum* producer of the carcinogenic mycotoxine patulin, are inhibited by all fractions assayed. (Paster et al. 1995). To evaluate their possible commercial use, both common food preservatives and the *Ng* fractions were tested in conditions used for preservatives. The activity of these SEs was similar to those of propylparaben and orto phenylphenol (Table 2).

CONCLUDING REMARKS

The morphological diversity of the trichomes of the two plants is also expressed in the chemical composition of their SEs and the antifungal activity of these secretions. The fungal inhibition is of the same level as that of common commercial food preservatives. In general, the compounds from *Ng* are more active than those from *Ss*. In spite of this, the only natural SEs described in the literature capable of inhibiting the growth of *A. flavus* are M2 and M3 from *Ss*. The other mycotoxigenic fungi, patulin producing *P. expansum*, is inhibited by all SEs tested. These results have two possible uses: the use of SEs extracts as natural antifungals in postharvest treatments, mainly in "organic farming", and as source of new germplasm with antifungic properties, that could be used to introduce these resistance

characters in Solanaceae (Pragnell, 2003) crops, such as potatoes, tomatoes or aubergines.

ACKNOWLEDGMENTS

Bachs. Horacio Pezzaroglio and Daniel Lorenzo are great fully acknowledged for taking NMR and GC-MS spectra and Bach. Gabriel Gonzalez for the technical assistance in the graphic design.

REFERENCES

CESIO, M.V.; HEINZEN, H.; MONTEIRO DE BARROS, N. and MOYNA, P. Characterization and biological activity towards the soybean caterpillar *Anticarsia gemmatallis* of new sugar esters from Solanum sisymbrifolium (Lamark). In: *IUPAC International Symposium on the Chemistry of Natural Products* (22º, 3rd-8th September 2000, Sao Carlos, Brazil) Abstracts 2000 p. 173. Sao Carlos, Editorial de la UFSCAR, 2000.

GARCÍA, S.; GARCÍA, C.; HEINZEN, H. and MOYNA, P. Chemical basis of the resistance of Barley seeds to pathogenic fungi. *Phytochemistry*, 1997, vol. 44, no. 3, p. 415-418.

GOFFREDA, J.C.; MUTSCHLER, M.A.; AVÉ, D.A.; TINGEY, W.M. and STEFFENS, J.C. Aphid deterrence by glucose esters in glandular trichome exudate of the wild tomato, *Lycopersicon pennelli*. *Journal of Chemical Ecology*, 1989, vol. 15, no. 7, p. 2135-2147.

HARBORNE, JEFFREY B. Twenty-five years of chemical ecology. *Natural Products Reports,* 2001, vol. 18, no. 4, p. 361-379.

JUVIK, J.A.; SHAPIRO, J.A.; YOUNG, T.E. and MUTSCHLER, M.A. Acylglucoses from wild tomatoes alter behaviour and reduce growth and survival of *Helicoverpa zea* and *Spodoptera exigua* (Lepidoptera: Nostuidae). *Journal of Economic Entomology*, 1994, vol. 87, no. 2, p. 482-492.

KENNEDY, B.S.; NIELESEN, M.T.; SEVERSON, R.F.; STEPHENSON, M.K. and JACKSON, D.M. Leaf surface chemicals from *Nicotiana* affecting germination of *Pernospora tabacina* (ADAM) Sporangia. *Journal of Chemical Ecology*, 1992, vol. 15, no. 9, p. 2135-2147.

KING, R.R.; CALHOUN, L.A. and BOUCHER, A. Sucrose esters associated with glandular trichomes of wild *Lycopersicon* species. *Phytochemistry*, 1990, vol. 29, no. 7, p. 2115-2118.

KOLATTUKUDY, P.E.; ROGERS, L.M.; DAOXIN, L.; HWANG, C.H. and FLAISHMAN, M.A. Surface signaling in pathogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, May 1995, vol. 92, no. 1, p. 4080-4087.

LARRAMENDI, S.; BARRA, C.; HEINZEN, H. and MOYNA, P. Influence of seed epicuticular waxes from different sunflower cultivars on *Aspergillus flavus* growth and aflatoxin biosynthesis. In: SANCHEZ, J.; CERDÁ-OLMEDO, E. and MARTINES-FORCE, E., eds. *Advances in Plant Lipid Research*, Sevilla, July 1998, p. 609-612.

OHYA, I.; SHINOZAKI, Y.; TOBITA, T.; TAKAHASHI, H. and MATSUZAKI, T. Sucrose esters from the surface lipids of *Petunia hibrida*. *Phytochemistry,* 1996, vol. 41, no. 3, p. 787-789.

PASTER, N.; HUPPERT, D. and BARKAI-GOLAN, R. Production of patulin by different strains of *Penicillium expansum* in pear and apple cultivars stored at different temperature and modified atmospheres. *Food Additives and Contaminants*, 1995, vol. 12, no. 1, p. 51-58.

PITT, J.I. and HOCKING, A.D. *Fungi and food spoilage*. Gaithersburg, Maryland; Aspen Publishers. 1999, 593 p. ISBN 0-8342.1306-0.

PRAGNELL, M. Crop protection in the new millennium. In: VOSS, J. and RAMOS, G., eds. *Chemistry of crop protection*, Basel, Wiley-VCH Verlag, 2003, p. 7-17.

RAHALISON, L.; HAMBURGER, M.; HOSTETTMAN, K.; MONOD M. and FRENK, E. A bioautographic agar overlay method for the detection of antifungal compounds from higher plants. *Phytochemical Analysis*, 1991, vol. 2, no. 4, p. 199-203.

SEVERSON, R.F.; CHORTYK, O.T.; STEPHENSON, M.G.; AKEY, D.H.; NEAL, J.W.; PITTARELLI, G.W.; JACKSON, D.M. and SISSON, V.A. Characterization of natural pesticide from *Nicotiana gossei.* In: HEDIN, P.A., ed. *Bioregulators for Crop Protection and Pest Control*. Washigton D.C., ACS, 1994, p. 109-121.

SHAPIRO, J.A.; STEFFENS, J.C. and MUTSCHLER M.A. Acylsugars of the wild tomato *Lycopersicon pennelli* in relation to geographic distribution of the species. *Biochemical Systematics and Ecology*, 1994, vol. 22, no. 6, p. 545-561.

STEFFENS, JOHN C. Acyltransferases in Protease's Clothing. *Plant Cell,* August 2000, vol. 12, no. 1, p.1253-1256.

VAN DER HOEVEN, R.S. and STEFFENS, J.C. Biosynthesis and elongation of short-and medium-chain-length fatty acids. *Plant Physiology*, January 2000, vol. 122, no. 1, p. 275-282.