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Research article

Soil management type differentially modulates the metabolomic profile of olive xylem sap

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ABSTRACT

In conventional olive growing, frequent soil tillage strongly reduces the complexity and diversity of the agro-ecosystem. Here, a metabolomic analysis was carried out on the xylem sap (XS) of olive plants (*Olea europaea* L.) from a grove located in Southern Italy (Basilicata region). The orchard has been divided in two plots that have been managed for 18 years with two different systems: a) 'sustainable management' (S_{mng}), with no-tillage, fertigation and internal C-inputs (spontaneous weeds and pruning residues), and b) an adjacent rainfed 'conventional management' (C_{mng}), that included soil tillage and mineral fertilization. The XS was extracted from olive shoots in two sampling times (ST1: May; ST2: October) using a Sholander pressure chamber, and its metabolome analyzed by ultra-high performance liquid chromatography (UHPLC) coupled to a hybrid quadrupole-time-of-flight mass spectrometer (QTOF-MS). The discriminating compounds were 94 at ST1 and 119 at ST2, and 35 of them were in common between the two sampling times. The majority of the discriminating metabolites (73 on 94 at ST1, and 109 on 119 at ST2) were found at higher concentration in the XS of S_{mng} plants, compared to that of C_{mng} ones. Most of the discriminating metabolites found in XS (about 80%, both at ST1 and ST2) were involved in plant secondary metabolism, mainly for plant chemical defense, growth regulation and signal transduction. The most prevailing class of compounds included terpenoids, phytohormones, alkaloids, sterols/steroids, retinols/retinoids, tocopherols and carotenoids. For the first time, we have demonstrated that the XS of a tree crop significantly responds to a shift of soil management. Generally, the plants of the S_{mng} plot showed an up-regulated secondary metabolism. The results of our study encourage the use of a set of sustainable agricultural practices in a productive orchard, in order to enhance plant physiological status, increase yield quantity/quality, safeguard the environment and ameliorate human health.

1. Introduction

Olive (*Olea europaea* L.) represents one of the most important oil crops world-wide, which has characterized the Mediterranean landscape since ancient times. In 2017, on an area of 10.65 Mha, 19.27 Mt of olives were harvested world-wide (FAOSTAT, 2017). Considering the relevance of this crop for semi-arid Mediterranean agricultural lands, a sustainable approach in olive orchard management is essential for improving soil quality, health and fertility (Sofo et al., 2014). The advantages of the adoption of a sustainable soil management that includes no/minimum tillage, cover crop application, incorporation of grass and pruning residues into the soil, and correct pruning, has been extensively studied in olive groves. Such benefits include a high level of soil microbial genetic/functional diversity and complexity both in the soil and in the phyllosphere (Sofo et al., 2014; Pascazio et al., 2015), a faster C and N turnover (Pascazio et al., 2018), higher levels of soil organic carbon (SOC) (Montanaro et al., 2012) and soil water content (Celano et al., 2011), and better soil physical (Palese et al., 2014) and chemical characteristics (Sofo et al., 2010).

The interactions between a plant and the composition of its xylem sap (XS) are highly complex and dynamic (Alvarez et al., 2008; Carella et al., 2016). The number and amounts of compounds in XS depend on some key factors, such as plant genotype and physiological status, sampled organ and tissue growth stage, sampling period, availability of soil nutrients, soil water potential and soil environmental conditions (Dambrine et al., 1995; Carella et al., 2016). If XS composition can be affected by differential agricultural practices is still a matter of debate, but no definitive findings are present in literature. Some attempts were

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Abbreviations: PGP, Plant-growth promoting; ST, Sampling time; XS, Xylem sap

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carried out with *in vitro* experiments. For instance, Lu et al. (2009) found that different nitrogen forms (NO_3^- or NH_4^+ applied in different proportions) affect cytokinin (CK) content in xylem sap of tomato seedlings, and that zeatin riboside is the main responsible for plant growth. Yong et al. (2000) applied elevated CO_2 (720 ppm) coupled with low mineral nutrition (2 mM NO_3^-) and observed a significant increase of CK content in cotton plants grown in greenhouse. Besides CKs, XP is also vehicle for other plant growth regulators such as indole-3-acetic acid and its derivatives (Sorce et al., 2002), and abscisic acid (ABA) (Alvarez et al., 2008).

Xylem sap is important for the transport of photosynthate and N-compounds throughout the plant body (Carella et al., 2016) and it contains a wide number of carbohydrates, amino acids, and proteins of different types and functions (Krishnan et al., 2011). Furthermore, plants can change XS composition when attacked by a microbial pathogen through the increase in the concentration of several phenylpropanoids, some amino acids and alkaloids, and other defense compounds (mainly phenolics, but also pentaketides and α-glucan, pullulan and stilbenes) (Bruno and Sparapano, 2006; Alvarez et al., 2008).

According to our knowledge, the information on how tree XS metabolome is modified by a different management system of an orchard is lacking. Very few studies (e.g., Ferguson et al., 1983; Sorce et al., 2002; Bruno and Sparapano, 2006; Lima et al., 2017) reported information on partial or complete sap metabolome in tree crops, and none of these dealt with the effects of agronomic practices on XS composition. Nonetheless, recent advances in metabolomics and related bioinformatics offer the possibility to gain a rather comprehensive picture on the phytochemical profile in biological systems, including plants, thus opening new opportunities (Meier et al., 2017; Tsugawa, 2018). On this basis, the main aim of this study was to investigate the xylem sap phytochemical profile of olive plants grown for 18 years under two different management systems, namely sustainable (S_{mng}) and conventional (C_{mng}). We hypothesized that a sustainable management system (S_{mng}) could cause significant differences in XS composition. A deepening about XS metabolome of olive plants grown under different agronomic systems could be useful for investigating the presence of compounds with plant-growth promoting (PGP) properties, that could be beneficial to the plants and promote the quality of this important fruit crop.

2. Materials and methods

2.1. Experimental site and olive grove management

The trial was carried out in a 2-ha mature olive grove (*Olea europaea* L., cv. 'Maiatica'; plants with an age of approximately 70 years, trained to vase at a distance of 8 × 8 m; NE orientation) located in Ferrandina (Southern Italy, Basilicata region; N 40°29′, E 16°28′) and managed using organic agricultural practices (sustainable management, S_{mng}) since 2000. The area has a semi-arid climate. In 2017, the annual precipitation was 645 mm (46 mm in ST1 and 68 mm in ST2, the two sampling dates), and the mean annual temperature ranged from 15 to 17 °C. The soil of the experimental grove is a sandy loam, classified as a Haplic Calcisol (FAO, 2006). The soil has a low gravel content and shows an increasing concentration of finely divided calcium carbonate particles in the soil matrix passing from the surface horizons (0.0–0.5 m) to the parental material (soil layer > 0.6 m). The major landform is plain, the slope form is classified as convex-straight and the slope gradient class as gently sloping (2–5%) (FAO, 2006). The depth of the groundwater at the moment of the analysis was > 1.5 m.

In the S_{mng} plot, olive plants were drip-irrigated from March to October (2800 $m^3\,ha^{-1}\,year^{-1}$) with six drip emitters discharging 8 $L\,h^{-1}$ over a 1-m radius around each plant. The top 60-cm soil layer had an average pH of 7.62 ± 0.36 (SD), total organic carbon content of 10.82 ± 0.58 $g\,kg^{-1}$, total N equal to 1.48 ± 0.28 $g\,kg^{-1}$, and C/N of 6.99 ± 1.19, with a mean bulk density of 1.37 $t\,m^{-3}$. The average annual amounts of organic C, N, P and K distributed by the irrigation water were 124, 54, 30 and 50 $kg\,ha^{-1}\,year^{-1}$, respectively. An integrative amount of 40 $kg\,ha^{-1}$ of $N{-}NO_3^-$ per year was distributed by fertirrigation during fruit set and pit hardening phase, in order to entirely satisfy the nutrient needs of olive trees. Plants were lightly pruned every year in winter. The soil was permanently covered by spontaneous self-seeding weeds (mainly Fabaceae and Poaceae), mowed twice a year for avoiding competition for water and nutrients. Cover crop residues and shredded prunings were shredded and left along the row as mulch.

An adjacent plot, characterized by soil and trees having similar features, was taken as control and conducted with a locally conventional management (C_{mng}), according to the practices usually adopted by farmers in the region. The top 60-cm soil layer had an average pH of 7.97 ± 0.31 (SD), organic carbon content of 9.78 ± 0.20 $g\,kg^{-1}$, total N equal to 1.05 ± 0.12 $g\,kg^{-1}$, and C/N of 9.32 ± 1.40, with a mean bulk density of 1.22 $t\,m^{-3}$. The C_{mng} plot was managed by tillage (milling at 10 cm depth) performed 2–3 times per year to control weeds. Severe pruning was carried out every two years, and pruned residues were removed from the olive orchard. Irrigation with aqueduct water was conducted empirically by the farmers, only if needed. A mineral fertilization was carried out once per year, in early spring, using ternary compounds (NPK 20-10-10 fertilizer at doses ranging from 300 to 500 $kg\,ha^{-1}\,year^{-1}$).

There were no diseases nor biotic stresses (e.g., drought, excess heat), nor N and P deficiency symptoms in the trees of both the management systems. There were no differences in tree height (about 4.0–4.5 m) and diameter between S_{mng} and C_{mng} plots, whereas yield in 2017 was 7.8 $t\,ha^{-1}$ in the S_{mng} plot and 4.3 $t\,ha^{-1}$ in the C_{mng} one.

2.2. Sap collection and extraction

The xylem sap (XS) was extracted from shoots of olive trees for both the management systems in May 2017 (sampling time 1: ST1) and in October 2017 (sampling time 2: ST2). In order to avoid border interferences, plants located in the central part of each plot and far 24 m each other were randomly chosen. For each treatment, three replicates ($n = 3$) of XS, each from one single plant, were collected using a Sholander pressure chamber (Model 600, PMS Instruments, Corvallis, OR) pressurized with N_2. This method was chosen because the application of pressure did not cause rapid sap changes with time and minimized the contamination of the extracted sap by cellular contents.

Two shoots, approximately 15–20 cm in length, were taken from each of the four cardinal points per plant using sterile cutting shears. The plant material was put in plastic bags, transported to the laboratory and stored 4 °C before use. For each shoot, a 1-cm wide bark strip was removed in the proximal part with a sharp knife sterilized with 75% ethanol, in order to exclude the phloem sap and to prevent external contamination. The cut end of the stem was placed in the pressure chamber facing out. The foliage of the cutting was placed in the pressure chamber and the lid was locked down. Then, high pressure was applied (approximately from 5.0 to 7.0 MPa, *i.e.* 50–70 bar) to force plant XS from the tissue at the proximal end of the cutting. After discarding the first drops, XS was collected into Eppendorf tubes for 15–20 min per shoot and centrifuged at 12,000 × *g* for 10 min at 4 °C. Thereafter, supernatants were taken and kept at −80 °C until metabolomic analysis.

2.3. Metabolomic analysis

Each individual XS sample was diluted in 10 vol of 1% HCOOH in 80% methanol, then filtered through a 0.22 μm disposable cellulose membrane into an amber vial for analysis. The untargeted screening of metabolites in XS was carried out through an ultra-high performance liquid chromatography (UHPLC) system coupled to a hybrid quadrupole-time-of-flight mass spectrometer (QTOF-MS) using previously the method described by Rouphael et al. (2016) with small modifications.

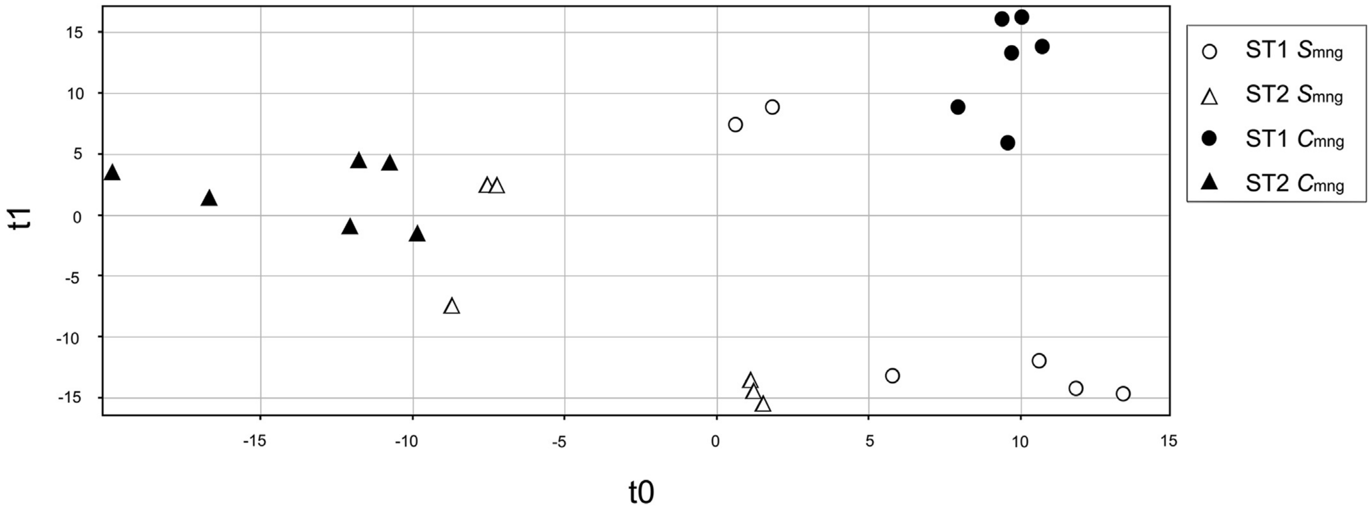

Fig. 1. Score plot from Partial Least Squares Discriminant Analysis (PLS-DA) multivariate analysis carried out from xylem sap metabolomic profile of olive plants grown either under sustainable (S_{mng}, white symbols) and conventional (C_{mng}, black symbols) soil management and at two different time points (ST1: May, circles; ST2: October, triangles).

The system included a 1290 liquid chromatograph, a G6550 iFunnel Q-TOF mass spectrometer and a Dual Electrospray JetStream ionization system (all from Agilent technologies, Santa Clara, CA, USA). Briefly, reverse phase chromatographic separation was achieved using an Agilent Zorbax Eclipse-plus C18 column (100 × 2.1 mm, 1.8 μm) and a mobile phase linear gradient (5%–95% methanol in water, 34 min run time) flowing at 220 μL min^{-1} at 35 °C. The mass spectrometer was run in positive ionization SCAN mode in the range of 100–1000 m/z^+. Each extract underwent a duplicate injection, and samples were randomly positioned in the analytical sequence; a blank (80% v/v aqueous methanol) was run after every three samples injection, whereas injection needle was washed for 8 s in 5% (v/v) HCOOH and 80% (v/v) acetonitrile following each injection.

Deconvolution and post-acquisition processing were done in Agilent Profinder B.06 using the 'find-by-formula' algorithm. After mass and retention time alignment, compounds annotation was achieved based on monoisotopic accurate mass (threshold $<$ 5 ppm), isotopes spacing and isotopes ratio. With this aim, the database PlantCyc 12.5 (Plant Metabolic Network, http://www.plantcyc.org; released in April 2018) was used. Those compounds having an annotation score of > 75/100 were maintained in the dataset. Thereafter, a filter-by-frequency post-processing filter was applied to retain only those compounds that were present in 66% of replications within at least one treatment. Based on the strategy adopted, the identification was carried out according to Level 2 (putatively annotated compounds) of COSMOS Metabolomics Standards Initiative (http://cosmos-fp7.eu/msi).

The classification of the differential compounds into biochemical classes was carried following PubChem (NCBI, https://pubchem.ncbi.nlm.nih.gov/) and PlantCyc information.

2.4. Statistical analysis

The interpretation of metabolomic data ($n = 3$) was carried out using Mass Profiler Professional B.12.06, as previously described (Salehi et al., 2018). Briefly, compound abundance was Log_2 transformed and normalized at the 75th percentile and baselined against the median. Thereafter, a supervised multivariate analysis, Partial Least Square Discriminant Analysis (PLS-DA, N-fold validation) was also carried out. An accuracy threshold of 100% was adopted following N-fold validation. Variables loading plot, *i.e.* the weights used to build the PLS-DA class prediction model, were then displayed according to their importance within the latent vectors, and the most relevant ones (VIP - Variables of Importance in Projection) were exported from the covariance structures in the PLS-DA hyperspace.

Finally, analysis of variance (unpaired *t*-test, $p < 0.01$, Bonferroni multiple testing correction) and fold-change analysis (fold-change cut-off = 5) were combined into Volcano Plot pairwise comparisons. Venn analysis on Volcano-filtered compounds allowed identifying the differential compounds shared between the two time points from those exclusive of a single time point.

3. Results and discussion

3.1. Xylem sap extraction and discriminating metabolites

Because of the difficulty and laborious sap extraction technique, and to the high-tension gradient and very low leaf water potentials (up to −7.5 MPa in leaves) of olive plants (Dichio et al., 2006), limited amounts of XS were extracted from each shoot and the procedure was time-consuming. Thus notwithstanding, the method here used allowed to extract enough amounts of XS for the following metabolomic analysis.

For a long time, XS has been considered as containing only water and minerals, but various studies have successively demonstrated that it also contains metabolites and proteins (Mazzafera and Gonçalves, 1998; Sorce et al., 2002; Alvarez et al., 2008; Lu et al., 2009; Krishnan et al., 2011; Carella et al., 2016). In fact, more than 800 compounds were found in the XS of plants from either S_{mng} and C_{mng} plots. The whole list of compounds revealed in XS is provided as supplementary material, together with their abundances and composite spectra (mass and abundance combinations) (Supplementary Table S1).

Starting from this wide phytochemical profile, PLS-DA modelling allowed to classify samples into four groups, thus confirming a seasonal variability in XS composition and indicating that soil management has distinctively shaped XS metabolome (Fig. 1). Indeed, accuracy was 100%, with no misclassification following training and validation of the PLS-DA class prediction model. On this basis, the combination of ANOVA and fold-change analysis from Volcano Plot was used to identify differential metabolites, *i.e.* those differentiating the two management systems. Differential compounds were 94 for ST1 and 119 for ST2. Among these compounds, 35 were in common between the two sampling times, while 59 were found only at ST1 and 84 only at ST2 (Fig. 2). Most of the common metabolites were terpenoids with different numbers of carbon (C) atoms (mono-, di- and triterpenoids; 12 on 35), fatty acid compounds with a different degree of saturation (5), alkaloids (3), indole derivatives (3) and retinols (2). At ST1, 73 of the

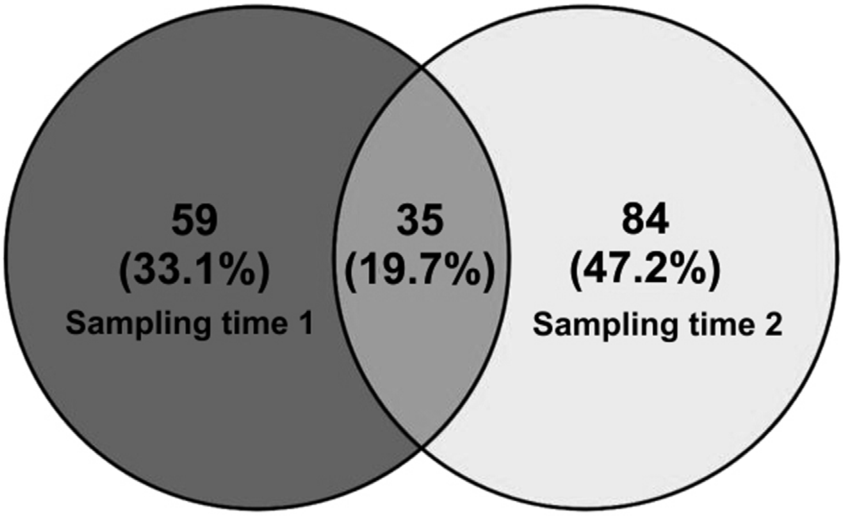

Fig. 2. Venn analysis from differential metabolites (as provided by Volcano Plot analysis, at $p < 0.01$ and fold-change > 5) in xylem sap, discriminating olive plants grown either under sustainable (S_{mng}) and conventional (C_{mng}) soil management at two different time points (ST1: May; ST2: October).

94 discriminating metabolites were found at higher concentration in the XS of S_{mng} plants and only 21 higher in that of C_{mng} plants (Table 1). At ST2, 109 of the 119 discriminating metabolites were found at higher concentration in plants from the S_{mng} plot and only 10 higher in those from the C_{mng} plot (Table 2).

3.2. Main classes of discriminating metabolites

Despite the discriminating metabolites belonged to heterogenous biochemical classes, a clear trend was observed. Indeed, only few compounds were primary metabolites, including some amino acids and amino acid-derivatives (8 detected at ST1 and 6 at ST2), as well as glycerol and fatty acid-derived compounds (16 at ST1 and 18 at ST2). Generally, the primary metabolism was only partially affected by the soil management adopted, as amino acids derivatives were generally over-represented in the XS of S_{mng} plants (Tables 1 and 2). Pascazio et al. (2018) have recently found that the irrigation method and soil management can deeply affect nitrogen (N) cycling in a sustainable olive grove, highlighting the contribution of soil bacteria in N transformations. In the XS, these change in N dynamics are likely reflected by different NO_2^-/NH_4^+ and asparagine contents (Mazzafera and Gonçalves, 1998), while other secondary metabolites, such as cytokinins and their purine-based precursors (Yong et al., 2000; Lu et al., 2009), alkaloids (Mazzafera and Gonçalves, 1998), and amino acids deriving from polypeptide hormones (Krishnan et al., 2011), can be more intensively regulated by different growth environments.

Regarding fatty acids derivatives, they were generally over-represented in the XS of S_{mng} plants, even if some cases of down-accumulation were observed (6 in ST1 and 2 in ST2). Interestingly, diacylglycerols at both ST1 and ST2 were down-represented in the XS of S_{mng} plants (Tables 1 and 2). This could reflect a change in hormonal balance, as these substances are not only membrane constituents and storage lipids, but also signaling lipids and precursor of phosphatidic acid (PA), with a pivotal role in the plant's response to environmental signals, through the PLC (phospholipase C)-DGK (diacylglycerol kinase) pathway (Arisz et al., 2009). This is supported by the fact that phosphatidyl compounds deriving from PA (1-16:0-2-lysophosphatidylcholine and 1-oleyl-2-lyso-phosphatidate at ST1, and 1-18:0-2-18:3-phosphatidylethanolamine at ST2) were higher in the XS of S_{mng} plants than in that of C_{mng} ones (Table 1).

Most of the discriminating metabolites (about 80%, both at ST1 and ST2) were involved in the secondary metabolism. Among them, the most prevailing class included terpenoids having different number of C atoms (25 at ST1 and 43 at ST2), phytohormones/plant growth regulators and their conjugates and derivatives (8 gibberellins, 5 auxins, 2 jasmonates, 2 cytokinins and 1 strigolactone at ST1; 4 auxins, 3 jasmonates, 1 gibberellin and 1 strigolactone at ST2); alkaloids (6 at ST1 and 12 at ST2); sterols/steroids (13 at ST1 and 2 at ST2); and retinols/retinoids, tocopherols and carotenoids (2 at ST1 and 6 at ST2) (Tables 1 and 2).

Plant terpenoids (or isoprenoids) are a large and diverse class of hydrocarbons composed of isoprene units. Unlike terpenes, from which they derive, they contain additional functional groups, usually with oxygen atoms, and most have multicyclic structures. Many plant terpenoids are repellant, due to their taste and smell, and/or toxic for a wide range of insects and fungi, so acting as a defense against herbivores, pathogens and parasites (Heldt et al., 2005; Singh and Sharma, 2015). The terpenoids with a direct effect on fungi are also included among 'phytoalexins' (Singh and Sharma, 2015) and were found to be more abundant in the XS of S_{mng} plants (kauralexin A2 at ST1; oryzalexin A, B, D, E, F, and kauralexin A1 at ST2) (Tables 1 and 2). Important monoterpenoid derivatives (10 C atoms) involved in plant defense against insects, such as a limonene-1,2-diol and (6*E*)-8-hydroxygeraniol, and (6*E*)-8-oxogeranial and a 7-hydroxy-4-isopropenyl-7-methyloxepan-2-one were found more abundant in the XS of S_{mng} plants (Tables 1 and 2). Other discriminating terpenoids are involved in phytohormone pathways (e.g., *trans*-abscisic alcohol for abscisic acid, 13-desoxypaxilline for indole-based auxins at ST2), carotenoids biosynthesis (prephytoene diphosphate at ST1), and sesqui- (15 C), di- (20 C) and triterpenoid (30 C) biosynthetic patterns. Most of them showed higher concentrations in plants subjected to a S_{mng} (Tables 1 and 2).

Many other plant secondary metabolites are biologically produced from terpenoid precursors, such as carotenoids and gibberellins (both have geranylgeranyl pyrophosphate as a precursor), steroids (from farnesyl pyrophosphate), cytokinins and quinones (Singh and Sharma, 2015; Lacombe and Achard, 2016). It is well known the antioxidant and protective action of carotenoids and their related (retinoids and retinols), even if their trend was not particularly clear, as they were up-accumulated in the XS of S_{mng} plants at ST2 but not at ST1 (Tables 1 and 2). Saponins, a class of glycosylated steroids, act as strong toxins against herbivores and fungi (Heldt et al., 2005). Oleanolate 3-β-D-glucuronoside-28-glucoside, medicagenate and 16-α-hydroxygypsogenate differentiated S_{mng} (higher levels) from C_{mng} plants at ST1 (Table 1), and all of them are precursors of saponins. Plant sterols are an essential component of cell membranes but, most interestingly, many of them act as

Table 1
Differential metabolites as provided by Volcano Plot analysis (*t*-test at $p < 0.01$ with Bonferroni multiple testing correction and fold-change > 5) in xylem sap metabolomic profile of olive plants grown under sustainable (S_{mng}) *vs* conventional (C_{mng}) soil at time point ST1 (May). Compounds are grouped in biochemical classes. The regulation (up or down) refers to the S_{mng} compared to C_{mng}.

Class	Compound	Regulation
Sterol lipids	porifersta-5,7-dienol	up
	avenasterol	up
	24-methylenelophenol	up
	4-alpha-14-alpha-dimethyl-9-beta-19-cyclo-5-alpha-cholest-24-en-3-beta-ol	up
	4-alpha-methyl-5-alpha-ergosta-8,24-dien-3-beta-ol	up
	porifersta-8,25(27)-dienol / porifersta-7,25(27)-dienol	up
	4-alpha-14-alpha-dimethyl-5-alpha-cholesta-8,24-dien-3-beta-ol	up
	17-alpha;-hydroxyprogesterone	up
	4,4-dimethyl-5-alpha-cholesta-8,14-dien-3-beta-ol	up
	4,4-dimethylzymosterol	up
	11-deoxycorticosterone	up
	isofucosterol	up
	Delta24-25 sitosterol	up
Other lipids	decanoate	up
	1-oleyl-2-lyso-phosphatidate	up
	2-R-hydroperoxy-linolenate	up
	(9Z)-12,13,17-trihydroxyoctadeca-9-enoate	up
	(9Z)-12,13-dihydroxyoctadeca-9-enoate	down
	dimorphecolate	down
	a 1-acyl-sn-glycero-3-phosphoglycerol (n-C14:1)	up
	1,3-dioctanoylglycerol	up
	10,16-dihydroxypalmitate	up
	18-oxo-oleate / 9,10-epoxy-12-cis-octadecenoate	down
	1-16:0-2-lysophosphatidylcholine	up
	1-18:2-2-16:3-monogalactosyldiacylglycerol	down
	1-18:3-2-16:2-monogalactosyldiacylglycerol	down
	1-18:3-2-16:3-monogalactosyldiacylglycerol	down
	1-18:3-2-trans-16:1-phosphatidylglycerol	up
Terpenes	a limonene-1,2-diol	up
	dehydroabietadiene-diol	down
	oryzalexin A	down
	3-beta-hydroxy-12,15-cassadiene-11-one	down
	9-beta-stemod-13(17)-en-19-oate	down
	kauralexin A2	up
	(6-E)-8-hydroxygeraniol	up
	heliocide B1/B2/B3/B4	up
	delta-tocotrienol	down
	oleanolate 3-beta-D-glucuronoside-28-glucoside	up
	medicagenate	up
	oleanolate 3-beta-D-glucuronoside	up
	glycyrrhetinate	up
	16-alpha-hydroxygypsogenate	up
	1,2-dihydrovomilenine	up
	13-desoxypaxilline	up
	3-hydroxyretinol	down
	prephytoene diphosphate	up
Gibberellins	gibberellin A15 (open lactone form)	up
	16-alpha- 17-epoxy gibberellin A12	up
	gibberellin A15 (closed lactone form)	up
	gibberellin A53	up
	gibberellin A110	up
	methyl gibberellin A20	up
	methyl gibberellin A4	up
	gibberellin A14	up
Jasmonates	(-)-jasmonoyl-L-isoleucine	up
	iso-jasmonoyl-L-isoleucine	up
Auxins	indole-3-acetyl-leucine / indole-3-acetyl-isoleucine	down
	indole-3-acetyl-phenylalanine	up
	alpha-naphthaleneacetamide	up
Cytokinins	isopentenyladenine-7/9-N-glucoside	up
Others	(+)-secoisolariciresinol diglucoside	up
	6-decylubiquinone	up
	N6-methyl-L-arginine	down
	8-methylthiooctylhydroximoyl-cysteinylglycine	up
	S-7-methylthioheptylhydroximoyl-L-cysteine	up
	4-(3-methylbut-2-enyl)-L-abrine	up
	homoarginine	down
	methoxydihydrosorgoleone	up
	9-mercaptodethiobiotin	up
	tylactone	up
	geissoschizine	up
	beta-fenchocamphorone	up
	1-phenyl-7-(3,4-dihydroxyphenyl)-hepta-1,3-dien-5-one	up
	(6S)-hydroxyhyoscyamine	up
	thebaine	up
	bestatin	up
	bornane-2,5-dione	down
	rhizobactin 1021 core	down
	cohumulone	up
	heptanal	up
	(iS/i)-nicotine	up
	chanoclavine-I	up
	aurachin C	up
	(+)-vernolate	down

secondary messengers, phytohormones regulating plant development (e.g., brassinosteroids), or defense substances (e.g., phytoecdysones) having a structure similar to that of the insect hormones. Our results demonstrated that all the discriminating steroids/sterols were more represented in the XS of S_{mng} plants at both ST1 and ST2 (Tables 1 and 2). Many of them are intermediates in the steroid biosynthetic patterns. Interestingly, ecdysone is an insect hormone with a steroid structure that controls the pupation of larvae. Plants can mimic these hormones, so that when insects eat plants containing phytoecdysones (triterpenoids), the pupation process is disturbed and the larvae die (Tarkowská and Strnad, 2016). It is hard to understand if the detected ecdysone was produced by insects (e.g., transmitted in the sap by aphid stylet or other insects) or if it was plant-synthesized. This compound was found to be more abundant in S_{mng} plants at ST1 (Table 1).

The physiological and phenological status of a plant is determined by its hormonal balance (Korovetska et al., 2016; De Ollas et al., 2018). The long-distance movement of many phytohormones from root to photosynthetic tissues through the xylem has been demonstrated (Lacombe and Achard, 2016), particularly for cytokinins (Yong et al., 2000; Lu et al., 2009), abscisic acid (Korovetska et al., 2016), but also for gibberellins, jasmonates, strigolactones and brassinosteroids. In particular, the biosynthetic patterns of many phytohormones are complex, intertwined and partly overlapping with those of many others primary and secondary metabolites (Heldt et al., 2005). The adoption of a S_{mng} caused an increase in some classes of phytohormones, and their conjugates and precursors, such as cytokinins (isopentenyladenine-7-*N*-glucoside and isopentenyladenine-9-*N*-glucoside), strigolactone precursors (tylactone at ST1 and carlactone at ST2), many gibberellins (especially at ST1), and jasmonates [(−)-jasmonoyl-L-isoleucine at both ST1 and ST2, and a jasmonoyl-1-aminocyclopropane-1 carboxylate at ST2) (Tables 1 and 2). All these phytohormones, excluding jasmonates that have mainly a defensive action, are secondary metabolites that in small amounts promote and regulate plant growth, development and differentiation of cells and tissues and, for this reason, they are also called "plant growth regulators" with PGP properties (Korovetska et al., 2016; De Ollas et al., 2018). A different case is that of auxins, whose transport is not mediated by xylem vessels (Lacombe and Achard, 2016). In our research, indole-3-acetic (auxin) precursors and degradation products were found in the xylem but never the final products (Tables 1 and 2). Interestingly, an auxin-like compound (*α*-

Table 2

Differential metabolites as provided by Volcano Plot analysis (t-test at $p < 0.01$ with Bonferroni multiple testing correction and fold-change > 5) in xylem sap metabolomic profile of olive plants grown under sustainable (S_{mng}) *vs* conventional (C_{mng}) soil at time point ST2 (October). Compounds are grouped in biochemical classes. The regulation (up or down) refers to the S_{mng} compared to C_{mng}.

Class	Compound	Regulation
Lipids	alpha-linolenate	up
	pinolenate	up
	gamma-linolenate	up
	2-omega-hydroxy-C22:0-LPA	up
	2-R-hydroperoxy-linolenate	up
	heptanoate	up
	(9Z)-12,13-dihydroxyoctadeca-9-enoate	up
	calendate	up
	alpha-eleostearate	up
	punicate	up
	dimorphecolate	up
	16-sinapoyloxypalmitate	up
	18-oxo-oleate	up
	1-18:0-2-18:3-phosphatidylethanolamine	up
	9,10-epoxy-12-cis-octadecenoate	up
	1-16:0-2-18:2-digalactosyldiacylglycerol	down
	1-18:2-2-16:0-digalactosyldiacylglycerol	down
	crepenynate	up
	ecdysone	up
	4-alpha-carboxy-4-beta,14-alpha-dimethyl-9-beta-19-cyclo-5-alpha-ergost-24-en-3-beta-ol	up
Terpenes	4,4'-diapolycopenedial	up
	levopiramadiene-diol	up
	dehydroabietadiene-diol	up
	neoabietadiene-diol	up
	dehydroabietadienol	up
	abieta-7,13-dien-18,18-diol	up
	palustradiene-diol	up
	isopimaradiene-diol	up
	neoabietadienal	up
	levopimaradienal	up
	palustradienal	up
	abieta-7,13-diene-18-al	up
	isopimaradienal	up
	9-beta-pimara-7,15-dien-19-al	up
	3-beta-hydroxy-12,15-cassadiene-11-one	up
	9-beta-stemod-13(17)-en-19-oate	up
	9-beta-stemod-13(17)-en-19-al	up
	ferruginol	up
	cyclooctatin	up
	kauralexin A1	up
	oryzalexin B	up
	oryzalexin D	up
	oryzalexin E	up
	oryzalexin F	up
	oryzalexin S	up
	oryzalexin A	up
	taxa-4,11-diene	up
	8-oxogeranial	down
	a 7-hydroxy-4-isopropenyl-7-methyloxepan-2-one	down
	geranylacetone	up
	heliocide B1	up
	heliocide B2	up
	heliocide B3	up
	heliocide B4	up
	oleanolate 3-beta-D-glucuronoside-28-glucoside	up
	glycyrrhetinate	up
	hederagenin	up
	alpha-curcumene	down
	heliespirone B	up
	abscisic alcohol	up
	germacra-1(10),4,11(13)-trien-12-al	up
	4-(5,5-dimethylcyclohex-1-en-1-yl)cyclohex-1-ene-1-carbaldehyde	up
Retinols	3-hydroxyretinol	up
	a retinol	up
Jasmonates	a jasmonoyl-1-aminocyclopropane-1 carboxylate	up
	jasmonoyl-L-isoleucine	up
	7-iso-jasmonoyl-L-isoleucine	up
Auxins	alpha-naphthaleneacetamide	down
	indole-3-butyryl-glucose	up
	10,11-epoxy-3-geranylgeranylindole	up
	13-desoxypaxilline	up
	indole-3-acetyl-phenylalanine	up
Phenolics	1-O-feruloyl-betaD-glucose	up
	1-phenyl-7-(3,4-dihydroxyphenyl)-hepta-1,3-dien-5-one	up
	coniferyl acetate	down
	taxa-4(20),11-dien-5-alpha,13-alpha-diol	up
	a rotenoid	up
	a 4'-methoxyisoflavone	up
Others	8-methylthiooctylhydroximoyl-cysteinylglycine	up
	S-7-methylthioheptylhydroximoyl-L-cysteine	up
	1,2-dihydrovomilenine	up
	geissoschizine	up
	carlactone	up
	1,7,9-tetramethylurate	up
	1,3,7,9-tetramethylurate	up
	2-methylpropanal-oxime	up
	Methyl-beta-D-glucoside 6-phosphate	up
	S-tetrahydroprotoberberine	up
	ent-kaurenal	up
	N-hydroxypentahomomethionine	up
	littorine	up
	N-caffeoylputrescine	up
	dethiobiotin	up
	3-geranyl-4-hydroxybenzoate	up
	piperazine-2-carboxamide	up
	7-methylthioheptanaldoxime	up
	13-hydroxylupanine	up
	hydroxyhyoscyamine	up
	thebaine	up
	N-isopropylformamide	up
	4-methyl-5-(beta-hydroxyethyl)thiazole	up
	1,2-di-S-octyl-1,2-dimercapto-3-propanol	up
	spinosyn tricyclic macrolactone	down
	11-dehydro-15-oxo spinosyn macrolactone	down
	bornane-2,5-dione	down
	rhizobactin 1021 core	up
	(S)-nicotine	up
	raucaffrinoline	up
	chanoclavine-I	up
	aurachin C	up
	juvenile hormone III	up
	dimeric urushiol peroxide	up
	(+)-vernolate	up

naphthaleneacetamide at ST1) and a degradation product of abscisic acid (*trans*-abscisic alcohol at ST2) were over-represented in the XS of S_{mng} plants (Tables 1 and 2). On the other side, jasmonates are partly xylem-borne hormones that have a PGP action (e.g., root elongation) and regulate stomata opening, but they also trigger both local and systemic defense responses (Lacombe and Achard, 2016; De Ollas et al., 2018).

Among the remaining compounds many free and conjugated phenols (1 at ST1 and 9 at ST2), lactones (3 at ST1 and 3 at ST1), purines (2 at ST2), oximes (3 at ST2), and other less represented compounds (lignans, carboxamides, alkanes/alkenes) were found at different concentrations between S_{mng} and C_{mng} plants. Among these compounds, phenols are likely the most important for plant defense and can be affected by different agronomic practices and environmental conditions, even if not always univocally (Sofo et al., 2016; Heimler et al., 2017). The S_{mng} provided soil with higher organic N and lower mineral N, and this likely was the reason of the increase in N-containing secondary metabolites (e.g., alkaloids and purines) and phenols, whose biosynthesis is generally induced when less nitrogen fertilizer is added to the soil (Heimler et al., 2017). Notably, flavonoids are also reported to modulate phytohormone signaling thus playing a functional role in plant-environment interactions (Brunetti et al., 2018).

Finally, other discriminating compounds between S_{mng} and C_{mng} plants included bacterial siderophores (rhizobactin 1021 core, produced by *Rhizobium* spp., at both ST1 and ST2), insect hormones and/or plant hormone-like compounds [(6 S)-hydroxyhyoscyamine at ST1, and ecdysone and juvenile hormone III at ST2], fungal fatty acids (crepenynate at ST2), natural insecticides produced by bacteria (spinosyn tricyclic macrolactone and 11-dehydro-15-oxo spinosyn macrolactone at ST2), intermediate products of camphor [e.g., β-fenchocamphorone at ST1 and (+)-bornane-2,5-dione at both ST1 and ST2], and biotin (9-mercaptodethiobiotin at ST1 and dethiobiotin at ST2).

3.3. Overview of the effects of soil management and sampling time on xylem sap composition

The majority of the discriminating compounds were found at significantly higher concentrations in the XS of S_{mng} plants, with the exception of some compounds of the following classes: retinols (ST1), indole-3-acetic acid conjugates (ST1), monoterpenoids (ST2) and spinosyn metabolites (ST2). From the overall data it emerges that the adoption of a S_{mng} for a long term determined a better plant status in terms of chemical defenses (e.g., terpenoids, alkaloids, phytoalexins, jasmonates, phenols), PGP phytohormones (e.g., gibberellins, cytokinins, strigolactones) and cell functionality (e.g., vitamin A, biotin, steroid second messengers). A S_{mng} including cover crops and internal C-inputs likely increased microbial diversity (Sofo et al., 2010, 2014; Pascazio et al., 2018), causing the presence of microbial-derived compounds beneficial for plants (siderophores, hormone-like substances, antibiotics).

Regarding the sampling time (ST1 and ST2), the 35 common compounds showed in the Venn diagram (Fig. 2) included mostly terpenoids (13), jasmonates (2), amino acids (2), alkaloid (3), being down-accumulated in the XS of S_{mng} plants. However, some steroids and retinols had an opposite trend at ST1 and ST2 (Tables 1 and 2). Interestingly, excepting for some auxin compounds (α-naphthaleneacetamide and 13-desoxypaxilline), hormones were not shared between the two sampling times (Tables 1 and 2). This suggests that the vegetative stage of olive plants played a pivotal role in determining the actual phytohormones profile in the XP, as already reported for auxins and abscisic acid by Sofo et al. (2018).

4. Conclusions

A sustainable orchard management is a key factor for enhancing soil fertility, *i.e.* the ability to supply the nutrients essential to plant growth (Sofo et al., 2010; Celano et al., 2011; Montanaro et al., 2012). Two additional key aspects are linked to soil fertility, namely: a) soil quality, *i.e.* the capacity of a soil to function within ecosystem boundaries to sustain biological productivity and promoting environmental, plant and animal health; and b) soil health, *i.e.* the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans. According to previous studies carried out in olive agro-ecosystems sustainably managed (Sofo et al., 2010, 2014; Pascazio et al., 2018), our data support that also xylem sap significantly responded to a shift of soil management toward a sustainable olive growing. The results of this study encourage the adoption of a set of sustainable agricultural practices (e.g., grass cover, pruning residues recycling, organic matter inputs) able to enhance plant physiological status, growth and natural defenses, with additional benefits on yield quantity/quality, the environment and human health.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.04.036.

Author contributions

AS, CF and AM designed and carried out the research. CF extracted the xylem sap from the trees. LL carried out the metabolomic analyses. LL and BD analyzed the data. AS and LL wrote the manuscript. All authors read and approved the manuscript.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Table S1. Whole dataset on xylem sap metabolomic profile of olive plants grown either under sustainable (Smng) and conventional (Cmng) soil management and at two different time points (ST1: May; ST2: October). Individual abundances are provided together with composite spectrum (mass and abundance combinations) of each compound annotated.

Compound	Conventional						Sustainable						Conventional						Sustainable							
	May												October													
	Sap_3_II-r001	Sap_3_II-r	Sap_4_II-r	Sap_4_II-r	Sap_5_II-r	Sap_5_II-r	Sap_C_II-r	Sap_C_II-r	Sap_D_II-r	Sap_D_II-r	Sap_E_II-r	Sap_E_II-r	Sap_3_III-	Sap_3_III-	Sap_4_III-	Sap_4_III-	Sap_5_III-	Sap_5_III-	Sap_C_III-	Sap_C_III-	Sap_D_III-	Sap_D_III-	Sap_E_III-	Sap_E_III-	Retention Time	CompositeSpectrum
7-oxatyphasterol	162158	160353	142816	1	1	156114	1	189849	165118	153304	161209	136639	157069	166291	1	1	1	1	181258	1	161762	168613	1	181573	23.76	(448.3492, 9357.9375)(449.35352, 1195.5508)(469.36325, 339.38333)(469.36423, 348.54333)(474.3503, 355.07)(470.39594, 838.80884)
1-18:3-2-16:0-monogalactosyldiacylglycerol	259895	246036	220492	224053	237075	246222	277102	286085	239575	240044	220625	240368	251317	236278	224717	218009	239912	260418	225567	228903	217944	249069	217645	199076	29.96	(757.52606, 10344.538)(758.5289, 1200.4908)
citronellate	1	347151	1	1	1	1	1648075	1551272	839218	944527	1	1	298888	763534	1	166493	508843	514616	2049165	2128147	1	1	328007	1175693	10.22	(155.12871, 20120.389)(156.133, 2202.74)(169.14783, 69305.55)(170.15503, 9114.143)(193.11525, 3146.619)
(12Z,15Z)-9,10-epoxyoctadeca-12,15-dienoate	166552	157654	1	1	1	1	1	1	1	230812	1	123857	176614	114828	161721	117806	1	1	154641	128737	1	1	1	135635	1.85	(296.88437, 1481.3433)(275.20364, 47079.875)(276.20978, 7931.872)(277.20233, 1844.0166)(297.89566, 802.6567)(298.2305, 1827.28)(277.214, 4584.74)(279.2146, 256216.1)(280.218, 46418.07)(281.2252, 8822.07)(311.24673, 1887.4532)(303.01993, 2869.34)
cinchonine	788159	1060692	751679	701419	839511	883246	1168340	1277715	973273	963145	299540	473227	969572	1140273	366361	426866	741682	727395	1865755	1969792	555553	528494	788828	984126	1.83	(295.18353, 551.3163)(277.16965, 30987.21)(278.1731, 2226.3865)(299.5308, 1246.341)(281.18774, 1076.1019)(314.07684, 746.65857)(298.8517, 1194.36)(299.41714, 1363.344)(300.21753, 850.70496)(322.18246, 153624.45)(323.18375, 20246.377)(303.42108, 1992.3604)(304.22504, 6139.0337)(305.1757, 10079.013)
cinnamoyltyramine	1035873	1193443	361206	338304	608173	642065	692788	711300	797039	793533	215639	460033	336183	455438	329407	459397	554406	565882	838242	873148	256202	249080	493827	750134	1.76	(268.1299, 361.44)(249.11308, 20290.064)(250.11703, 604.805)(251.1079, 1043.82)(272.8678, 568.50885)(253.12988, 503.252)(288.67194, 8685.213)(289.90262, 7057.012)(290.17606, 2487.2617)(271.1618, 1506.1133)(275.1167, 445.174)
parthenolide	913418	847226	1682440	1625973	1066797	1077324	602260	578741	824628	809884	1541839	1547635	1605266	1339808	1908317	1615419	1473346	1430577	925455	972022	1774077	1674611	1255264	1131733	1.93	(233.05302, 79185.7)(234.09846, 8679.537)(234.89558, 3754.38)(249.14984, 736.6988)(231.1374, 40307.99)(232.14203, 2680.8545)(267.17624, 437.2)(248.99101, 3863.6072)
iO/i-sinapoylcholine	257223	313421	371809	374189	434400	441931	181527	1	245071	246508	244740	372062	225499	319100	1	331381	340629	333736	178870	183070	386937	347667	201607	254114	1.73	(310.1631, 11849.946)(294.16068, 2987.0388)(296.1621, 1213.5616)(297.88925, 688.72626)(298.1728, 736.71)(333.21613, 2293.0898)(312.195, 1239.55)(335.16464, 39581.312)(318.90475, 1722.14)
(iR/i)-mandelonitrile	463211	832871	946535	961936	879618	884726	815965	840410	813419	806118	762595	876224	694525	757531	474689	896009	801038	845265	875204	829001	798666	466531	757463	807551	25.52	(151.08589, 32596.271)(152.0897, 607.2753)
cyclooctat-9-en-7-ol	1	1	1	1	1	2092332	2177938	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2235709	0.96	(274.2595, 6282.36)(290.25806, 101.479996)(291.27066, 25743.717)(292.2609, 65.75)(293.2578, 1314.73)
iN/i-acetyl-D-galactosamine	1.29E+07	1.47E+07	5602506	5216962	7841773	8064691	1.60E+07	1.62E+07	1.71E+07	1.63E+07	2805335	4668349	9828137	1.31E+07	8996660	1.23E+07	1.17E+07	1.17E+07	2.27E+07	2.38E+07	5475726	5320979	1.36E+07	1.87E+07	1.73	(222.09267, 23360.432)(240.46214, 446.16333)(221.11333, 599603.7)(223.11267, 46548.08)(224.11528, 2790.9673)(245.08391, 3000.84)
iN/isup1/sup-acetylspermine	440499	515787	518449	629515	524473	536246	499531	538927	429296	518573	416257	429396	439851	517888	425294	519627	544288	442287	530143	429304	618399	531715	536217	543046	20.49	(249.25655, 90721.57)(250.25626, 13304.338)(230.24446, 47379.332)(231.24333, 1362084.6)(253.23405, 15487.472)(255.23396, 25643.592)
marneral	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	371018	1	1	1	295614	11.28	(450.37485, 17104.336)(451.37854, 2970.355)
pentalenolactone D	1	1	1	1	1	1	135072	1	1	1	1	1	1	1	1	1	1	1	191324	175338	1	1	1	221909	2.06	(263.13016, 19492.057)(264.1358, 601.41003)(286.1746, 5008.173)(263.1502, 3208.13)
a carvone	122315	111405	1	1	1	1	128850	1	1	1	1	1	109734	1	106382	1	1	133362	1	1	1	1	128099	108595	26.33	(170.14215, 601.06)(150.12888, 13219.232)
2-heptyl-4(1iH/i)-quinolone	839918	1288494	512942	552112	795288	847003	1583901	1619476	914573	967961	1	1	1	282010	1	347849	1073628	1056216	2230002	2189949	1	520177	1298941	2384529	12.57	(243.16125, 57008.145)(244.16507, 6374.9526)(226.1515, 4607.2544)(227.1596, 381.95)(230.67429, 1337.255)(231.176, 3206.24)(244.18839, 832.546)
6-oxocampestanol	3464876	611584	9047344	5209533	2.06E+07	1	927718	523444	174527	479060	8403659	6703365	1	9031754	2.24E+07	2.36E+07	384449	2.62E+07	2.46E+07	1.98E+07	1300508	576389	8828392	669417	25.32	(421.37808, 1385.43)(398.35922, 35377.11)(420.58554, 1383.3461)(401.86993, 33700.824)(402.50565, 2240.4358)(436.5353, 547.64874)(436.4104, 286.11)(418.52142, 3460.3445)(419.9351, 1822.3108)(422.20612, 855.86505)(422.4044, 2027.61)(441.66534, 3131.3892)(441.3567, 2380.13)(442.3601, 377.17)(423.89987, 7548.508)(426.24704, 101
(6α)-hydroxycampestanol	264701	1	255350	1	271069	1	1	282674	400677	231482	210611	137740	215241	215427	1	286752	1	253677	1	287412	171071	1	359770	279165	16.07	(422.89218, 5865.7866)(424.062, 1234.1633)(403.38312, 2801.265)(406.38785, 5921.8237)(423.06216, 8789.775)(423.3968, 7230.07)(404.38544, 2768.837)(406.38858, 5403.0327)(437.41498, 5331.7256)(438.8595, 1482.2089)(439.43008, 2808.49)(440.4214, 133.84)(419.0931, 2799.418)(445.3871, 440.02)(428.3791, 178.44)
18'-hydroxy-χ,χ-caroten-18-oate	1	1	1	1	1	1	128709	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	135723	12.46	(574.33514, 7305.12)(575.3349, 696.79)(559.3384, 1383.14)(596.3782, 3751.34)(601.3338, 1792.605)
2-pentadecanone	550542	835415	284382	976237	296727	495392	1	531253	1	1	337086	721603	1	1117508	1	321770	1	353351	577072	642324	265252	1	1083090	705870	23.15	(227.40758, 181139.83)(208.91556, 17909.805)(209.22, 6388.55)(228.1226, 60663.613)(209.22429, 14461.3545)(253.23158, 24797.727)(231.20894, 13583.188)
lithocholate	626669	873690	647598	623953	1	486840	120096	672824	607299	540637	1	17108	1	356797	660805	1	561141	382372	749835	809703	1	691462	829164	788537	30.84	(379.2998, 262.7)(357.27576, 9336.059)(380.1005, 5438.0923)(381.03503, 19676.602)(381.31293, 23928.133)(363.30453, 70949.734)(394.43857, 1454.9438)(395.44135, 8057.3203)(396.3287, 10850.622)(375.36713, 22622.412)(377.3204, 8080.57)
χ,χ-caroten-18-oate	2011711	3261368	1355653	1373008	1101531	1101821	5949465	6301987	3427853	3563779	276995	605914	411101	812873	421219	785213	646155	619172	1004296	999244	154544	140107	383382	821526	10.22	(557.3606, 43681.83)(558.36285, 9631.726)(559.3653, 3044.4915)(584.3413, 12384.237)(585.3439, 3234.1704)(586.3579, 4581.005)(562.31696, 27013.93)(563.32007, 6445.5083)(564.3244, 2395.7961)
cathasterone	1	975149	4493438	4393029	1436185	1519351	914105	924386	1481586	1513616	869636	889942	1502611	1951573	888349	986706	1008402	1001505	912204	988220	1048221	902570	886305	1084020	13.29	(415.35724, 3683.3237)(433.3631, 807.885)(415.35535, 12651.489)(416.3552, 1017.27)(454.40625, 7310.005)(455.40466, 2379.575)(456.4084, 783.44495)(432.46997, 32924.56)(433.38608, 6703.5337)(434.38828, 1224.2949)(438.67838, 2119.1567)(440.34225, 3390.76)(443.36285, 4975.81)
(-)-ephedrine	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.28E+07	3271724	1	1	1.51E+07	15.35	(165.11507, 522983.5)(166.11842, 51658.47)(167.12256, 6332.98)(149.1165, 1306.9934)(184.15294, 7699.4233)(185.15483, 10944.27)
7-dehydrodesmosterol	1	1915361	820673	562411	173705	2135262	360459	1386295	171716	541962	406132	382761	624660	2730501	514831	691276	642741	207162	1	1	342985	557218	285954	428344	24.17	(386.2308, 21836.693)(387.34363, 51224.5)(388.33957, 98041.414)(364.31403, 12094.991)(384.66992, 19623.264)(384.34393, 7070.0903)(365.9435, 6021.226)(367.31577, 13043.904)(369.3288, 59868.67)(401.45425, 17099.385)(403.364, 16904.092)(406.3739, 524.5)(383.18912, 9785.277)(405.31226, 4730.1284)(406.3145, 781.86)(389.0226, 185
4α-hydroxymethyl,4β,14α-dimethyl-9β,19-cyclo-5α-ergost-24(24sup1/sup)-en-3β	1258253	1889612	829339	1	1447056	1	1221413	281158	399466	728671	355459	745350	1	892077	396670	1015238	672610	1171630	720214	488089	970376	2742526	339481	680868	27.58	(458.2399, 9446.382)(459.26544, 6166.6313)(461.07996, 2301.8152)(439.62042, 6285.998)(440.84872, 8815.653)(441.85422, 8984.882)(442.40042, 12006.715)(443.9005, 213.375)(458.3084, 11582.229)(459.5244, 4973.8877)(461.16772, 2712.254)(440.39423, 11099.82)(441.39752, 9558.03)(442.40112, 8680.122)(443.40128, 301.77002)(474.4353
thalianol	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	371018	1	1	1	295614	11.28	(450.37485, 17104.336)(451.37854, 2970.355)
(iS/i)-nicotine	1	1	1	1	1	1	898216	904838	161383	163996	1	1	1	1	1	1	1	1	1	1	1	1	986776	202397	13.20	(163.121, 844.82495)(164.13092, 13344.6455)(165.13463, 832.79)
2-ω-hydroxy-C22:0-LPA	1	200649	1	1	1	1	506322	527826	225788	241046	1	1	1	1	1	1	1	1	1	1	1	1	1	140771	11.16	(508.3146, 14213.278)(509.31845, 2441.7517)(519.3124, 361.53497)
iN/i-carbamoylputrescine	271418	142782	1	106509	1	1	990568	437355	322091	333764	1	1	1	1	1	1407233	244151	89015	1	1422991	1	477296	276284	525461	17.45	(115.10477, 6908.7812)(136.12894, 1183.8099)(118.11718, 52148.707)(150.1472, 7010.824)(151.1535, 142.94499)(132.13547, 50066.37)(133.13892, 721.77246)(157.1089, 533.18)(139.0955, 840.78)
4-prenylphloroisovalerophenone	660009	675512	706492	699021	599386	808065	885445	1012845	923894	956233	311009	489687	969572	1140273	373960	426866	518507	727395	1850615	1483898	456835	543848	385790	977105	1.83	(278.1435, 180.51)(259.63654, 1752.6613)(260.13116, 650.31)(283.1681, 709.24)(262.33832, 1406.6766)(263.15018, 2077.9763)(265.15396, 628.1475)(277.16965, 31585.297)(278.17307, 2304.5127)(300.13632, 1167.9419)
(3iS/i)-2,3-epoxy-2,3-dihydrosqualene	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	371018	1	1	1	295614	11.28	(450.37485, 17104.336)(451.37854, 2970.355)
oleanolate 3-β-D-glucuronoside-28-glucoside	1	1	1	1	1	1	143424	164770	130615	136304	1	1	1	1	1	1	1	1	162585	175188	1	1	1	150799	14.97	(794.4679, 2063.797)(799.42395, 6028.0083)(800.4273, 1117.8866)
feruloylagmatine	269482	367550	1	1	496251	520508	718384	744598	267523	347473	1	1	172150	194024	130376	129711	234798	238427	811027	578921	167067	145678	244304	162484	5.55	(291.9749, 2981.5898)(308.18494, 10277.86)(309.189, 1858.8351)(291.25012, 3058.4126)(327.212, 2214.17)(330.2277, 1440.5)(309.55844, 22500.898)(310.02795, 5868.42)(335.17603, 10951.263)
4α, 14α-dimethyl ergosta-8,25(27)-dienol	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	371018	1	1	1	295614	11.28	(450.37485, 17104.336)(451.37854, 2970.355)

iN/i-acetylneuraminate	284919	357140	577718	566643	336404	346158	424081	423973	356115	356709	1	302762	220415	386552	395315	652605	533948	527813	635066	679811	447576	420223	444777	757002	1.75
leukotriene-C4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	179998	183166	315736	304572	1	1	191257	377458	9.13
(i22R,23R/i)-28-homocastasterone	695523	1146910	518334	520988	435731	446101	4095010	1809446	2057313	2202670	174180	360968	1092365	2184346	469557	864028	1674949	1678236	4968561	5148375	523552	515596	1230871	3045026	10.06
(12iS/i)-HPETE	1	1	253828	262960	1	1	1687380	1856198	1421586	1406355	1	1	1	1	1	1	91885	1	1	1	1	1	1	226809	6.43
methyl gibberellin Asub20/sub	1	1	1	1	1	1	405090	395499	195789	234098	1	1	1	99818	1	1	1	1	464893	427991	1	1	1	145634	1.91
8β-hydroxy-germacra-1(10),4,11(13)-trien-12-oate	913418	847226	1696587	1625973	1107558	1103284	602260	578741	844231	809884	1551368	1565999	1620479	1357748	1901845	1615419	1482380	1438123	903370	969426	1891607	1756977	1302397	1143485	1.93
2,3-dimethyl-6-phytyl-1,4-benzoquinol	1	611584	1	1	1	1	1	523444	1	1	1	1	1	1	1	1	1	1	1	1	1	576389	1	669417	11.44
heliannuol C	913418	847226	1682440	1625973	1066797	1077324	602260	578741	824628	809884	1541839	1547635	1605266	1339808	1908317	1615419	1473346	1430577	925455	972022	1774077	1674611	1255264	1131733	1.93
hopan-22-ol	4456591	4383605	4319594	4391098	4470034	4454138	4033907	4010285	4517102	4026249	4662556	4706637	4560935	4419634	4309242	4304957	4437984	4127149	4324283	4402186	299291	285478	4607935	4501199	16.70
3,5-dihydroxy-6,7-didehydro-12'-apo-β-caroten-12'-al	117957	204150	1	1	1	1	1085537	1095062	376739	420632	1	1	224874	454568	1	165412	288030	302457	782817	1	1	1	136082	437843	7.23
palustradienal	254216	344487	1	1	1	1	670722	354121	537675	601621	1	1	1	1	1	1	1	1	431093	448368	1	1	354794	633134	11.52
3-methyl-1,2-didehydro-2,3-dihydrosqualene	117158	118138	107102	117077	128635	127043	126004	137014	121449	116350	1	116870	114527	114995	116493	122161	117492	114149	131148	125370	120966	120245	110173	107510	26.25
methionine iS/i-oxide	281325	269549	176857	1	1	1	191816	160683	280092	233683	1	1	381885	371813	316979	217453	313312	332624	198671	159113	377630	293409	298958	225891	1.58
tricyclene	6152430	6220118	6209308	6199382	6384860	6462884	6154197	6510099	6325897	1	6434756	6336232	1	1	6065257	6307751	6410170	6418699	6160912	6424674	6417830	6144594	6315134	5341283	31.03
(iZ/i)-β-ocimene	6152430	6220118	6209308	6199382	6384860	6462884	6154197	6510099	6325897	1	6434756	6336232	1	1	6065257	6307751	6410170	6418699	6160912	6424674	6417830	6144594	6315134	5341283	31.03
hexadecanedioate	262757	299280	306693	114142	344522	239732	387814	428560	481625	238153	1	1	482639	582047	370527	346336	864454	609539	875130	329818	781200	658812	1717612	1054331	2.20
canthaxanthin	3288608	4472867	4999816	5323745	1821995	1798344	3780234	3920239	4281359	4444112	2768039	4641163	2652287	3881599	2564912	3945251	4069938	4074291	4095323	4113179	3930748	3906859	2159765	3646396	23.15
3-dehydroteasterone	1	162270	153049	1	1	165274	1	466943	165118	153061	169845	145166	896900	1581458	214045	167242	188344	1	181258	524493	1	177890	198858	298738	20.38
5-(hydroxymethyl)-2-methyl-4(1H)-pyrimidinone	5341187	5270997	9993460	7516301	5776615	6283518	5405983	5196494	5705038	5382356	7560007	9251605	4847247	5484331	5536947	7032868	6940727	6711438	6083702	6546250	8780709	8362490	4814531	6471349	1.59
sinefungin	1248100	1595935	393394	417724	720557	642906	1856133	1741591	2003622	2044731	121440	283733	764802	1282584	672994	870297	1054839	1067055	2508811	2644421	315898	360789	1213589	1890394	1.77
a 1-acyl-sn-glycero-3-phosphoglycerol (n-C14:1)	1	1	1	1	1	1	224336	239876	1	94521	1	1	1	1	1	1	115225	115743	372388	388149	1	1	140652	474665	12.57
capsanthin	521831	963337	2045133	846345	252781	177839	503618	546633	292388	503278	211233	369212	299041	375537	241029	338238	392848	710494	607789	423605	604301	621390	264654	577860	23.15
miraxanthin V	569957	577863	539928	570906	575964	602769	561456	595259	573129	572921	567099	585932	553820	546486	548221	571017	558845	586356	572955	583917	582877	586305	570294	591800	21.67
(13E)-11-α-hydroxy-9,15-dioxoprost-13-enoate	876544	1120750	765340	705919	751025	756884	1288906	1302951	1682149	1705571	392078	642475	1924376	2308248	1389846	1585898	2491563	2431305	4792757	4905061	1454002	1382658	3850760	4733476	1.87
desmosterol	1	1	726887	1	732108	1	1	3789091	1	3809159	1113246	1	1	1	1	1	1	1	1295935	1	1	269835	1	3061753	22.92
11-deoxoglycyrrhetinate	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2057112	1	1	1	1	527881	1	1	1	2466178	25.97
L-iN/isup6/sup-methyl-L-arginine	1	1	170356	220381	400538	448919	1	1	1	1	1	1	116968	123413	157346	164118	171651	186370	117192	122936	137145	145764	125785	139028	1.75
deferoxamine	437306	581873	317570	311898	263159	284386	4288647	894250	1594212	1682779	203388	458002	278778	489097	413687	688118	315910	312883	451709	472846	127906	112590	218528	379722	14.61
iN/i-acetyl-α-D-glucosamine	1.29E+07	1.47E+07	5602506	5216962	7841773	8064691	1.60E+07	1.62E+07	1.71E+07	1.63E+07	2805335	4668349	9828137	1.31E+07	8996660	1.23E+07	1.17E+07	1.17E+07	2.27E+07	2.38E+07	5475726	5320979	1.36E+07	1.87E+07	1.73
cholestanol	383511	403913	174926	196076	9953270	9747224	312364	257747	130342	146673	9102729	245550	207629	221557	1	9906378	399886	427044	8796127	1	1	1.02E+07	443170	9432982	12.14
(9Z)-12,13,17-trihydroxyoctadeca-9-enoate	1	1	1	1	1	1	128068	124585	364998	378743	1	1	237283	295876	169816	1	360485	369902	616083	619566	1	1	509326	700204	3.52
16,17-dihydro-16α,17-dihydroxy gibberellin Asub12/sub	6533237	5836707	6951957	2813878	3419234	8035649	1.41E+07	1	1.06E+07	1.15E+07	6397816	8219024	1.59E+07	1.78E+07	1.13E+07	1.31E+07	1.08E+07	1.52E+07	1.75E+07	1.43E+07	3119324	7832773	519368	1.69E+07	1.85
3,22-dimethyl-1,2,23,24-tetradehydro-2,3,22,23-tetrahydrosqualene	1	1	1	140987	1	1	1	146075	1	1	1	1	130768	140914	1	1	139605	148404	1	1	138693	135843	1	136053	16.19
1,2-dioctanoyl-3-methyl-1,2,3-butanetriol	575437	284564	584017	1	541713	1.17E+07	226868	526647	518231	525018	457100	593505	395844	469722	570747	569500	1	605361	610745	579397	8997442	1	560068	628175	29.88
astaxanthin	119857	181735	2215890	2303424	705812	706347	4821377	5087963	4061329	4361252	1	288797	480614	1523057	2504571	3.16E+07	3.22E+07	5.09E+07	5.16E+07	1.06E+07	1.04E+07	2.77E+07	5.86E+07		15.37
dehydro-D-arabinono-1,4-lactone	152960	160757	500024	431625	216001	239226	151490	155765	158793	154928	297573	450847	136915	156220	192644	257068	209189	210685	172082	191260	398758	368855	157815	211832	1.60
O(2),1,7,9-tetramethylurate	120537	121549	1	1	115147	115952	146759	140605	155762	179656	1	86826	1	1	1	1	1	1	141004	99211	1	1	1	130090	1.73
laurate	516882	1	1	1	1001588	569000	390226	217433	1	163495	1	1	1	263135	149581	1	1150730	527323	395260	1	1718505	1	994522	673584	25.80
a limonene	6152430	6220118	6209308	6199382	6384860	6462884	6154197	6510099	6325897	1	6434756	6336232	1	1	6065257	6307751	6410170	6418699	6160912	6424674	6417830	6144594	6315134	5341283	31.03
11α-hydroxy-β-amyrin	1	1890818	1884518	1680005	1969326	1059089	1	1	2712155	2218956	1	1251033	1482904	503465	1252144	1	2473685	1	1956506	1	1	353460	1	1774535	24.42
3-dehydro-6-deoxoteasterone	1	975149	1	1	1436185	1519351	914105	924386	1481586	1513616	869636	889942	1502611	1951573	888349	986706	1008402	1001505	912204	988220	1048221	902570	886305	1084020	11.59
6α-hydroxy-germacra-1(10),4,11(13)-trien-12-oate	913418	847226	1696587	1625973	1107558	1103284	602260	578741	844231	809884	1551368	1565999	1620479	1357748	1901845	1615419	1482380	1438123	903370	969426	1891607	1756977	1302397	1143485	1.93
palmitate	1859853	1349542	270572	230932	1	1931901	1	243457	1475887	731158	885371	390004	1	331002	1488361	1	1894490	2644289	245514	251492	901341	1655969	1	470456	18.57
L-ornithine	1	1	1	1	1	1	224347	253934	1	113201	1	1	1	140513	1	1	1	1	435609	419328	1	1	1	179067	10.22
α-tocopherol	5938726	1037471	4625852	1	1	1577639	1	1	1	1	1	2387850	1	1	3780534	1	1	1	1544492	2171732	1	5047967	1	3309964	31.16
pimeloyl-CoA	1	2218651	1696996	874004	530680	279411	578556	1	1	525372	1	1	1	1	991260	1.16E+07	5022706	555790	1718322	1286088	2425043	589439	404922	813777	2.38
sterone-ring	7950783	8482351	564672	8222864	8309964	3228006	8630270	7754263	529030	311133	3588370	1	467634	290868	491820	499089	257982	281450	1	7938298	522754	1	523162	488278	24.59
1,3,7,9-tetramethylurate	120537	121549	1	1	115147	115952	146759	140605	155762	179656	1	86826	1	1	1	1	1	1	141004	99211	1	1	1	130090	1.73
2-oleoylglycerol	3965209	5790013	4485215	3576470	2531873	1780320	524298	2233520	577615	706442	993243	441297	2461331	1030198	1	1806359	827009	357522	4911400	5497894	3374141	322854	4652521	2027258	23.57
1-palmitoleoylglycerol 3-phosphate	1096213	1759024	665222	667251	1159909	1232447	6078037	6205207	1841297	1950427	114791	291914	115987	1	339637	610536	1238688	1247155	1	1	521839	505458	1763203	4860241	9.94

10-deoxysarpagine	788159	975610	751679	708447	839511	891933	1122915	1320544	996954	991586	299540	473227	969572	1140273	366361	426866
adenosine	626535	891020	871321	825126	742833	588791	636841	665262	626259	400228	120906	318618	227309	512806	429925	689021
S-methyl-L-methionine	1	418108	732664	732096	545641	458106	360297	304261	355455	104617	1	180608	1	187964	1	400230
hypusine	8454826	9181772	9219718	9532114	8823269	8794602	1.12E+07	1.12E+07	1.15E+07	1.16E+07	7426904	8331296	7122785	7632997	7592639	9148667
1-oleyl-2-lyso-phosphatidate	1	1	1	1	1	1	1	1	1	1	1	170256	213161	308190	210755	153238
(S)-citramalate	152960	160757	500024	431625	216001	239226	151490	155765	158793	154928	297573	450847	136915	156220	192644	257068
a methylhexadecanoic acid	150452	30750	334900	1	314368	853260	1	812275	1	340460	2049933	195588	634119	1247428	271369	161332
calcitriol	1	1	1	62955	1090366	1	1	1	1119964	1	1	1	1	1	1	1
14-demethyllanosterol	1056458	1369125	1441139	1465616	608673	550503	1059066	1	1193337	1265461	771447	1360006	810510	1108607	836695	1051612
9-cis-10'-apo-β-carotenal	1	1	1	136657	1	138808	395318	383945	449667	502357	1	1	247955	369378	1	236579
(R)-citramalate	152960	160757	500024	431625	216001	239226	151490	155765	158793	154928	297573	450847	136915	156220	192644	257068
poriferasta-7,25(27)-dienol	1	1	1	1	1	1	1804398	1	2142151	1687198	1	2303617	2249935	2183839	2141089	1
(+)-β-pinene	6152430	6220118	6209308	6199382	6384860	6462884	6154197	6510099	6325897	1	6434756	6336232	1	1	6065257	6307751
pseudo-conhydrine	194645	153494	734534	712166	1571253	1704969	1336043	1412604	1742313	1753388	120026	1997951	171737	1	148702	141961
D-arginine	1	1	1	119300	119224	123646	563462	591976	341480	299827	1	1	146646	351395	1	177823
digiproside	1	1	1	1	1	1	1	1	1	1	1	1	118574	215748	1	1
L-methionine-(R)-(S)-oxide	281325	269549	176857	1	1	1	191816	160683	280092	233683	1	1	381885	371813	316979	217453
dimeric urushiol peroxide	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3-oxo-2-(cis-2'-pentenyl)-cyclopentane-1-octanoate	166552	157654	1	1	1	1	1	1	1	230812	1	123857	176614	114828	161721	117806
(+)-3-carene	6152430	6220118	6209308	6199382	6384860	6462884	6154197	6510099	6325897	1	6434756	6336232	1	1	6065257	6307751
4,4'-diapolycopenedial	409612	761256	341091	359654	563673	599233	782830	797286	1661977	1756855	1	123414	1	1	1	1
(3S)-3-hydroxy-L-arginine	435814	1	504923	391060	537863	545587	1	241112	1	1	220898	247618	199322	428089	172952	368264
D-glutamate	1	1	1	1	1	1	96889	1	1	102632	1	1	1	1	1	1
multiflorenol	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
(22α)-hydroxy-isofucosterol	997369	1464719	1358665	1499689	1	627496	1106599	1229872	1213188	1148458	805821	1296653	872561	1	1	917569
cannabichromenate	267994	365400	896223	904991	235917	229578	6105385	6222507	4312276	4595005	1	1	1	231918	1	227649
7-dehydrocholesterol	1	1	726887	1	732108	1	1	3789091	1	3809159	1113246	1	1	1	1	1
pyrithiamine	833279	1288094	510864	546208	793837	821845	1576765	1619476	894100	943834	1	1	1	281610	1	338690
hederagenin	1	144256	1	1	1	1	294755	297960	110824	105108	1	1	1	1	1	1
an icosadienoate	572515	625896	539928	436358	365457	769282	439644	391431	518986	278773	323225	249881	99447	493470	359996	588469
hygrine	501806	583503	157150	208182	469369	474921	1279568	1275690	607610	579695	1	204120	1	1	1	1
steviolmonoside	1	264014	1	1	1	1	249580	261721	204911	1	1	1	1	1	1	166334
conhydrinone	501806	583503	157150	208182	469369	474921	1279568	1275690	607610	579695	1	204120	1	1	1	1
1-18:2-2-16:1-monogalactosyldiacylglycerol	259895	246036	220492	224053	237075	246222	277102	286085	239575	240044	220625	240368	251317	236278	224717	218009
ginsenoside Rg1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
catharanthine	2431811	3071162	1	3434179	3144313	3111737	2258035	2018101	2387424	2512401	1	1	1368575	2280822	1960962	2582206
(22R,23R)-22,23-dihydroxycampesterol	1	975149	1	1	1436185	1519351	914105	924386	1481586	1513616	869636	889942	1502611	1951573	888349	986706
4-guanidinobutyrate	534946	778327	440899	414412	583834	575823	1574509	1608691	1098878	1127331	1	326863	675011	1102548	478644	745693
1,3-dioctanoylglycerol	1	1	1	96984	1	1	1	414196	1127346	1240804	1	1	1	1	1	1
kauralexin B2	1	1	1	1	1	1	1	1	229435	250828	1	1	1	1	1	1
4,4-dimethylzymosterol	1	1	1	1	1	1	1804398	1	2142151	1687198	1	2303617	2249935	2183839	2141089	1
chloramphenicol	1	101338	1	1	1	1	209894	206303	132097	135173	1	1	1	1	1	1
gibberellin A8	5705950	7078593	2900018	2856186	4251108	4294520	1.35E+07	1.35E+07	1.02E+07	1.01E+07	1479298	2785449	3853913	5931817	3449752	5026582
2,3-dimethyl-6-geranylgeranyl-1,4-benzoquinol	351893	383324	373430	291313	307869	348666	334481	318182	313652	337634	446471	360777	1	379263	312061	459458
methyl β-D-glucoside 6-phosphate	1	185719	1	1	112739	1	280129	271144	264750	274580	1	1	1	1	1	1
N-caffeoylputrescine	220718	400001	1	1	1	1	208278	245453	179954	129996	1	1	1	1	1	1
tetrahydropalmatine	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
riboflavin	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
(-)-heliannuol A	1	1	1	1	1	1	1	1	252045	268870	1	1	1	1	1	1
linoleate-group	1	237372	1	1315477	584714	112451	1266998	7184424	1198255	2701500	1358275	1369035	1935345	116066	1071569	1239700