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Influence of microwave frequency electromagnetic radiation on terpene emission and content in aromatic plants

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SUMMARY

Influence of environmental stress factors on both crop and wild plants of nutritional value is an important research topic. The past research has focused on rising temperatures, drought, soil salinity and toxicity, but the potential effects of increased environmental contamination by human-generated electromagnetic radiation on plants have little been studied. Here we studied the influence of microwave irradiation at bands corresponding to wireless router (WLAN) and mobile devices (GSM) on leaf anatomy, essential oil content and volatile emissions in *Petroselinum crispum*, *Apium graveolens* and *Anethum graveolens*. Microwave irradiation resulted in thinner cell walls, smaller chloroplasts and mitochondria, and enhanced emissions of volatile compounds, in particular, monoterpenes and green leaf volatiles (GLV). These effects were stronger for WLAN-frequency microwaves. Essential oil content was enhanced by GSM-frequency microwaves, but the effect of WLAN-frequency microwaves was inhibitory. There was a direct relationship between microwave-induced structural and chemical modifications of the three plant species studied. These data collectively demonstrate that human-generated microwave pollution can potentially constitute a stress to the plants.

Introduction

Aromatic plants represent an important resource for human nutrition, due to their valuable properties, including medicinal benefits (Bonjar, 2004; Wong and Kitts, 2006; Bakkali et al., 2008; Ortan et al., 2009; Cornara et al., 2009). Therefore, understanding their chemical composition and how the properties of aromatic plants are affected by key climate change factors as well as human-generated pollution are research topics of major interest.

The key property of aromatic plants is the presence of essential oils that play important roles in plants acting as direct defenses against pathogen and herbivore attacks (Rhoades, 1977; Lewinsohn et al., 1991; Fugmann et al., 1997; Reddy et al., 2001). The essential oils are very complex natural mixtures that consist of molecules produced through different secondary metabolic pathways, characteristically containing terpenoids, benzenoids and sometimes aliphatic compounds (Bauer et al., 1998; Eggersdorfer et al., 1998; Cheng et al., 2007; Bakkali et al., 2008).

Both the composition and content of essential oils has been shown to strongly depend on plant species and environmental conditions (Langlille and MacLean, 1976; Letchamo et al., 1996; Zabaras et al., 2002; Manzan et al., 2003). These aspects are relevant because plants in natural conditions as well as in agricultural fields are exposed to a plethora of abiotic and biotic stresses and the importance of several biological and environmental stresses is expected to increase in the future (Peñuela and Estiarte, 1998; Lobell et al., 2008; Craufurd and Wheeler, 2009; Jacob and Winner, 2009; Niinemets, 2010a,b).

The key abiotic stresses (Lobell et al., 2008; Craufurd and Wheeler, 2009; Jacob and Winner, 2009) of contemporary economical importance for plant growth worldwide are drought, heat, cold (chilling and freezing), high salinity, soil mineral deficiency and toxicity. Furthermore, diffuse environmental pollution, including air and soil pollution constitutes a major problem for agriculture

Abbreviations: VOC, volatile organic compounds; GLV, green leaf volatiles; WLAN, wireless router; GSM, mobile devices; TEM, transmission electron microscopy.

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and human health (Gauderman et al., 2004; WHO, 2004; Copaciu et al., 2013; Opriş et al., 2013). It was demonstrated that the blend of volatile organic compounds (VOC) emitted by aromatic plants under stress factors is complex (Rodrigues-Navas et al., 2012). The complexity of volatile emissions in species having specialized storage structures for volatiles results from the circumstance that there may be emissions directly coming from storage and de novo emissions independent of storage (Staudt et al., 1997; Niinemets et al., 2010a,b; Monson et al., 2012; Grote et al., 2013; Li and Sharkey, 2013).

Among the novel potential pollution sources is the enhanced use of mobile phones and wireless devices generating an exponentially increased level of electromagnetic radiation in the microwave range of radiation frequencies (1–100 GHz). There have been some studies on microwave effects on plants showing no significant effects, while others have demonstrated important modifications in plant functioning. Laboratory growth experiments in plants subject to magnetic fields demonstrated that plants were taller and heavier (Martínez et al., 2003). Likewise, germination of *Cicer arietinum* L. seeds and early development were enhanced upon exposure to a moderate magnetic field (Vashisth and Nagarajan, 2008). It has been shown that electromagnetic radiation at broadcast-frequency (0.2–30 MHz) altered the cellular contents of calcium and sulfur, effect associated with the power of radiation (Balmori Martínez, 2003), while in animal cells has been observed that microwaves (frequency of 147 MHz, amplitude-modulated at 16 Hz) can influence the intercellular communication through altering the functioning of the calcium channels (Balmori Martínez, 2003). Exposure to microwaves (frequency of 9.75 GHz and low intensity) of wheat (*Triticum aestivum*) plants has resulted in cytogenetic changes (Pavel et al., 1998; Balmori Martínez, 2003). Studies have also shown alterations in condensed chromatin distribution of meristem cells exposed to low magnetic fields (Belyavskaya, 2001, 2004). In general, these studies collectively suggest that the effects of electromagnetic fields on plants can be variable.

It is, however, unclear what the mechanism of low-energy microwave irradiation effects on plant is. While high energy microwave-radiation can break the chemical bonds (Caldwell et al., 1995; Barnes and Cardoso-Vilhena, 1996), the quantum energy of microwave radiation is low and mainly can have thermal effects, heating up selectively plant structures and possibly also alter the conformation of biomolecules, such as proteins, nucleic acids and membrane lipids. Furthermore, modifications in biomolecular tertiary structure can importantly alter the rate of physiological processes, again implying that microwaves can lead to stress conditions in plants (Takeuchi and Thornber, 1994; Ha et al., 1997; Havaux, 1998). Thus, it is important to gain more conclusive insight into the effects of microwaves on plant performance.

The aim of the present study was to investigate the influence of microwave irradiation on the ultrastructure of leaves, the essential oil content and VOC emission of three aromatic plant species of the Apiaceae family, parsley (*Petroselinum crispum* L.), dill (*Anethum graveolens* L. subsp. *hortorum* Alef.) and celery (*Apium graveolens* L.). The stress application consisted in three weeks microwave irradiation of plants at bands corresponding to wireless router (WLAN) and mobile devices (GSM). As the emissions of stress volatiles such as green leaf volatiles (GLV) and specific terpenes are enhanced upon exposure to different stresses (Heiden et al., 2003; Beauchamp et al., 2005; Copolovici et al., 2011; Niinemets et al., 2013), we hypothesized that microwave irradiation leads to enhanced emission of stress volatiles. In addition, we intend to investigate how microwave irradiation affects the leaf structure, content and composition of essential oils in these aromatic plants of nutritional and medicinal importance.

Materials and methods

Plant material and growth conditions

Plant material including parsley (*Petroselinum crispum* cv. Plain leaved 2) (P), dill (*Anethum graveolens* subsp. *hortorum* cv. Common) (D) and celery (*Apium graveolens* cv. Pascal Giant) (C) were grown in laboratory from seeds obtained from Agrosel (Câmpia Turzii, Romania). Fifteen seeds were sown in 150 mL plastic pots (height × diameter of 8.5 cm × 6.5 cm) filled with commercial garden soil.

Three weeks after seeding, the vessels with plants were placed in three identical anechoic chambers (Surducan et al., 2012) characterized by a degree of isolation of 60 dB at radio-frequency range between the exterior and interior. The fully-closed chambers were maintained under the same conditions of light intensity at 300 μmol m^{-2} s^{-1} provided from four 4 W MR16 LED lamps (every lamp consisting of 26 warm white SMD 5050 LED at 3300 K), temperature (25 °C), CO_2 concentration (385 ± 20 ppmv) and humidity (65%). One chamber was for non-treated control plants, while plants in the other two chambers were subjected to microwave irradiation. The microwave irradiation was performed at bands corresponding to mobile devices (GSM) using a modified AP5200 generator (D-LINK, China), operating in four bands (860–910 MHz frequency range, Pout 29 dBm), and to wireless router (WLAN) using a D-LINK wireless router 802.11 g/2.4 GHz (2.412–2.48 GHz frequency range, Pout 19 dBm). In the irradiation chamber there is one stick antenna placed in the center of the ceiling. The exposure levels where chosen in agreement with the microwave irradiation levels measured in open space for heavily used GSM networks (100 mW/m^2) and for indoor WLAN (70 mW/m^2) communication protocols. The power density to the base of chambers was measured with a spectrum analyzer SPECTRAN HF 4060, AARONIA AG (Germany). Both control and microwave-irradiated plants were watered every 2 days with 10 mL of bidistilled water (bidistillator AcquaMatic model AWC/4D, Hamilton Laboratory Glass Ltd., Kent, UK).

Irradiation was performed during three weeks, after which plants were removed from the chambers for measurements of volatile organic compound (VOC) emission and analyses of leaf structure and essential oil content. All measurements of VOC emission and analyses of leaf structure and essential oil content have been replicated with eight different plants.

Transmission electron microscopy measurements

Samples for transmission electron microscopy (TEM) were contrasted with 2% uranyl acetate in 50% ethanol solution for 2 min and in 0.2% lead citrate in 0.1 M sodium hydroxide solution for 2 min. The samples were dehydrated in ethanol series and embedded in epoxy resin, Epon 812. The samples were cut in an Ultramicrotome, Leica UC6 with a diamond knife and the ultrathin samples (100 nm) were analyzed with a 120 kV TEM Model JEM 1010 (Jeol USA Inc., Peabody, MA, USA). The number of chloroplasts, mitochondria, starch grains in the chloroplasts and nuclei were determined in the palisade mesophyll cells for replicate plants under each treatment.

Essential oil extraction

Samples of fresh plant material of 1 g were frozen in liquid nitrogen, pulverized and essential oils were extracted with 2 mL of 1:1 (v/v) mixture of HPLC-grade diethyl ether and *n*-hexane (Merck, Germany). For extraction, plant material was initially soaked for 10 min with the solvent mixture, and then extracted in an ultrasonic bath (Elmasonic S 15H, 37 kHz) for 30 min at 30 °C. Each extraction was performed using five parallel samples. In all cases, extracts