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Volcani Voice

Agricultural Research Organization –Volcani Center Annual E-Newsletter

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Message from the Editors

By Ada Rafaeli & Nirit Bernstein

ARO Women in the Agricultural Sciences

The Editorial Board of *Volcani Voice* decided to adopt this year's theme: *"A Time for Women – Achievements and Challenges*" as exemplified during the Independence Day Celebration in 2014, which included the traditional lighting of the beacons by women representing a wide spectrum in Israeli Society.

As a special tribute, and in recognition of their achievements at Volcani Center, Volcani Voice is dedicating this issue as a proud appreciation to the accomplishments of ARO Women in the Agricultural Sciences. The issue contains a select sample of the research accomplishments from our superb staff including 6 articles covering various themes that bear high importance to the agricultural sector.

Included are two articles from the Institute of Plant Sciences; one by **Nurit Katzir** (awarded the ***Lifetime Achievement Award, 2013***) and the second by **Hinanit Koltai**. An article from the Plant Protection Institute by **Victoria Soroker**; an article from the Soil, Water and Environment Institute by **Ellen Graber** (awarded ***Scientist of the Year, 2013***); an article from the Institute of Postharvest and Food Sciences, **Elena Poverenov** and an article from the Agricultural Engineering Institute by **Ronit Rud**.

The underlying genetic components of melon fruit quality traits are revealed using genomic resources

By Galil Tzuri[1], Vitaly Portnoy[1], Navot Galpaz[1], Shery Lev[1], Rotem Harel-Beja[1],Yael Danin-Poleg[2], Itay Gonda[1], Omer Barad[3], Doron Shem-Tov[3], Zhangjun Fei[4], Adi Doron-Faigenboim[5], Shiri Freilich[1], Merav Kenigswald[1], Ayala Meir[1], Einat Bar[1], Uzi Saar[1], Ya'akov Tadmor[1], Josef Burger[1], Efraim Lewinsohn[1], James J. Giovannoni[4] Arthur A. Schaffer[5], Nurit Katzir[1]

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About Nurit Kazir

Dr. Nurit Katzir is a senior research scientist in the Cucurbit Research Unit at Newe Ya'ar Research Center and a member of the Department of Vegetable Research of the ARO. Her research focuses on the genetics and genomics of melon (*Cucumis melo* L.) and related Cucurbit crops. Major topics of her research include fruit quality, genetic mapping and the assessment of genetic variability. Recently, her transcriptomic and metabolomic studies have resulted in a system that provides a highly accurate mapping that enables fast pinpointing of the causal genes that underlie fruit quality traits. The main goal of the research is to apply these genes to the improvement of cucurbit fruit quality. Dr. Katzir was head of the Newe Ya'ar Research Center (2005-2012) and received the Lifetime Achievement Award of the ARO, 2013.

The major goal of our research over the past two decades has been to identify novel genes underlying traits of agronomic importance, mainly those that are involved in metabolic pathways determining fruit quality in melon (*Cucumis melo*). A systems biology research strategy was applied, combining phenotypic, transcriptomic and metabolomic analyses of a recombinant inbred population (414 X Dul) derived from phylogenetically diverse parents. High throughput sequencing of the transcriptome (RNA-seq) of 94 lines of the population and their parents enabled (1) Identification of *ca*. 60,000 informative SNP markers for quantitative trait loci (QTL) and (2) Mapping of expression QTL (eQTL). Metabolite profiles were collected using both targeted (sugars, organic acids, carotenoids) and non-targeted metabolomic analyses. QTL analysis resulted in highly accurate mapping that enabled the pinpointing of the causal genes underlying major traits of interest. The results demonstrate the rapid technological and resource developments over the research period.

Melon (*Cucumis melo* L.)

Melon (*Cucumis melo* L.), belonging to the Cucurbitaceae family, is a highly polymorphic species, widely cultivated throughout the world. Melon fruits are most variable in their shape, size, rind characters, firmness, color and flavor. Fruits of wild and cultivated genotypes vary in their accumulation levels of soluble sugars, organic acids, pigments and aroma volatiles, affecting fruit quality through complex networks of metabolic pathways that are active during fruit ripening[1]. Furthermore, melon cultivars vary also in ripening physiology, disease resistance and additional traits.

A recombinant inbred lines (RIL) population (414 x Dul) was developed from a cross between representatives of the two melon subspecies: PI414723 *(Cucumis melo* subsp. *agrestis* var. *momordica*) and 'Dulce' (*C. melo* subsp. *melo* var. *reticulatus*) [4, 8]. The initial goal of the development of this population was to map genes associated with ZYMV resistance. The only known source of ZYMV resistance was the multi resistant line PI414723

Fortuitously, the parental lines of the population differ in numerous fruit quality traits as well, including sweetness, acidity and color, external rind characters, size, shape and firmness. This RIL population is, therefore, an excellent resource for the study of fruit quality traits. Figure 1 depicts the two parental lines of the population and a selection of RILs, demonstrating polymorphism among the lines and homogeneity within the lines.

"The high throughput transcriptome analysis enabled an ultra-high resolution and thus the fast identification of the underlying genetic components of melon fruit quality traits."

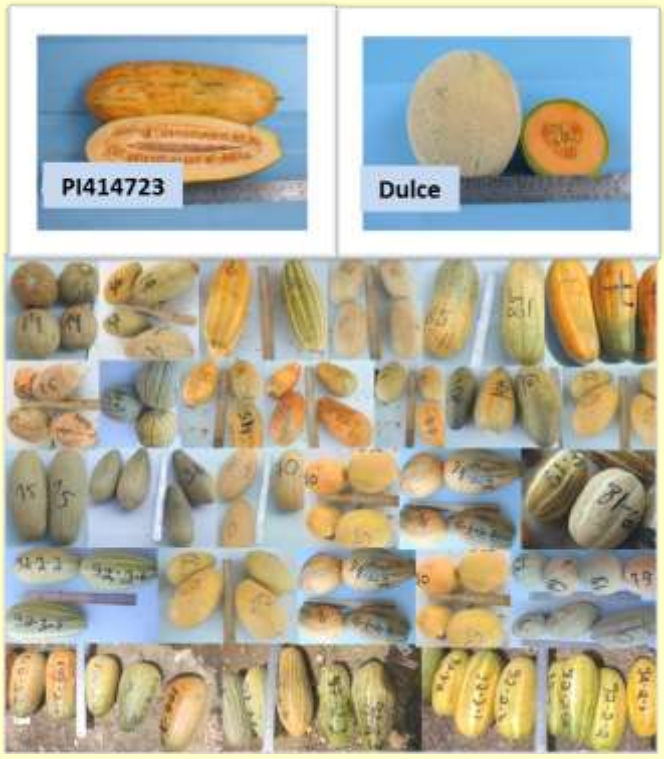

Figure 1: The parental lines of the 414 x Dul population: PI 414723 and 'Dulce' and a selection of RIL. Each square represents several fruits of the same line, demonstrating the polymorphism among the lines and the homogeneity within each line.

Genetic mapping

Genetic maps are powerful tools for linking genes and phenotypes. Most of the important agricultural traits are quantitative, and controlled by quantitative trait loci (QTL). QTL analysis is a potent tool for the detection of genomic regions that control specific traits enabling gene discovery and Marker Assisted Selection (MAS). QTL for fruit traits, such as total soluble solids (TSS), fruit size and shape, climacteric ripening, netting, color and various metabolites, including carotene, sugars and organic acids have been genetically mapped. Several of the melon genetic maps have been merged[5], including our 414 x Dul map[4, 8]. We have recently obtained a highly accurate QTL analysis with excellent resolution by use of the 414 x Dul RILs population and of transcriptomic and metabolomic analyses (Galpaz *et al.*, in preparation).

The rapid advancements in mapping and QTL analysis technologies are made apparent by our genetic mapping projects. The first 414 x Dul map was developed by phenotyping and genotyping of plants in the F_{2-3} generation. It contained five traits: ZYMV resistance (*Zym*-1), Fusarium resistance (*Fom*-1), female flower form (*a*= andromonoecious), striped epicarp (*st*), and fruit flesh pH (*pH*). All but *Fom* were mapped for the first time. In addition, 70 molecular markers were mapped, 22 of which were Simple Sequence Repeat (SSR) markers, known to be highly informative, locus-specific, co-dominant markers[4]. Notably, SSR markers linked to the *pH* and *Zym*-1 genes enabled the beginning of a successful yet long and tedious map-based cloning of these genes[3] (and Perl-Treves in preparation, respectively). Figure 2A depicts the map of linkage group 2 that includes the *Zym*-1 locus adjacent to its linked marker and the *a* gene, responsible for andromonoecy in melons.

The 414 x Dul population was further developed into an advanced RIL population by single-seed descendant propagation to create the population of 99 RILs comprising a mix of F_{6-8} generation lines. This population was used for the construction of an advanced genetic map, enriched for fruit traits and almost 700 markers[8]. Over two-thirds of the markers were co-dominant SSR, SNP and INDEL markers, nearly 200 of which were derived from fruit EST libraries. Candidate genes encoding for enzymes of sugar and carotenoid metabolic pathways were cloned from melon cDNA or identified through mining of the International Cucurbit Genomics Initiative database (http://www.icugi.org/).

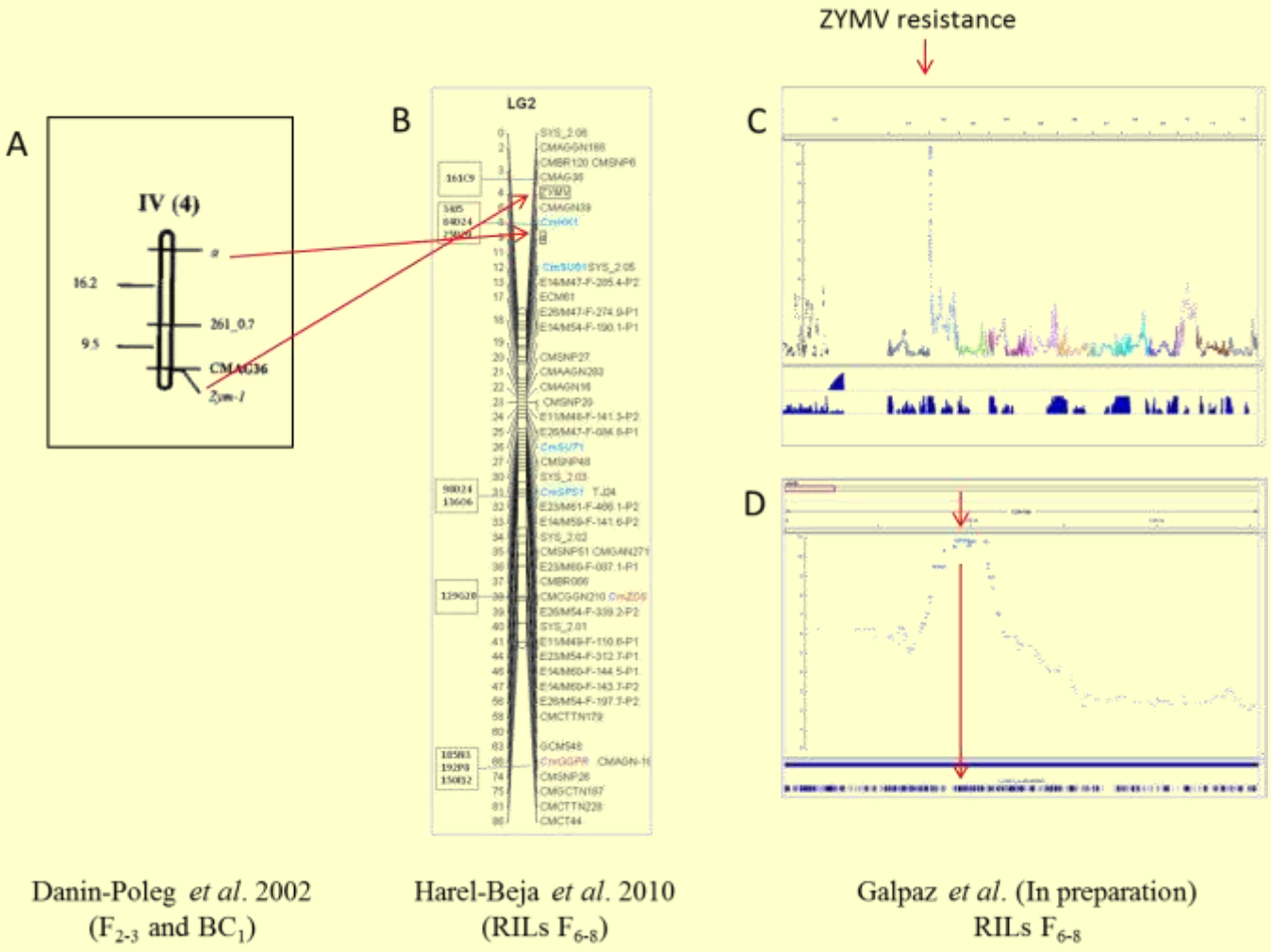

***Figure 2**: QTL analysis of the resistance to ZYMV. The Zym-1 locus on linkage group 2 (previously 4): (A) Danin-Poleg et al.; (B) Harel-Beja et al.; (C) IGV snapshot of the peak in the resolution of all chromosomes (each in a different color) and (D) zoom-in on chromosome 2. The blue lines below are the genes. The QTL interval was of 12 genes*

The map includes 44 fruit QTL for fruit sugar content, fruit flesh color and carotenoid content, viral resistance (ZYMV) and additional phenotypic traits (Figure 2B). Interestingly, no clear co-localization of QTL for either sugar, acid or carotenoid content was observed with nearly 100 genes encoding for enzymes involved in their metabolism[2 8].

Recently, a systems biology approach combining global phenotyping and genotyping of the 414 x Dul population led to QTL analysis at single gene resolution. Phenotypic and genotypic data collection included: (1) High-throughput next-generation sequencing of the transcriptome (RNA-seq) of mature fruit from 94 RILs and their parents; (2) Phenotypic and metabolic profiling of the same samples using targeted (HPLC analyses of sugars, organic acids and carotenoids and GCMS analysis of volatiles) and non-targeted metabolomic analysis (LCMS). QTL analysis was performed using *ca.* 60,000 SNPs derived from the transcriptome and mapped to the melon genome[7] resulting in highly accurate QTL detection of the phenotypic and metabolic traits. The analysis of traits for which previously cloned and functionally analyzed genes were available served for QTL validation. QTLs for fruit acidity, fruit shape and selected volatile levels were located within 1-9 genes of known genes that control them, demonstrating the

high resolution and accuracy of the mapping strategy. A novel, causal gene of a QTL of one of the volatiles was identified in the QTL interval and its function was proven. Figures 2C&D present the QTL of the resistance to ZYMV on chromosome 2. Zoom-in into the QTL interval of 12 genes enabled the identification of two resistance genes. Map based cloning by Perl-Treves *et al.*, (in preparation) resulted in the identification of the same two genes, proving again the high accuracy of the analysis. Notably, the comparison of the three maps (Figure 2) demonstrated the accuracy of all three, while the significant progress in resolution was achieved by use of the advanced RIL population and by the increased number of SNP markers. The high throughput transcriptome analysis enabled an ultra-high resolution and thus the fast identification of the underlying genetic components of melon fruit quality traits.

Novel candidate genes for additional fruit quality traits of importance were identified and are currently being validated and functionally analyzed. A large germplasm collection and additional segregating populations are available for preliminary validation. In addition, RNA profiles of all the genes expressed in the mature fruit were analyzed as quantitative traits, leading to the identification and mapping of over 10,000 eQTL. These are expected to reveal mechanisms affecting gene expression[6]. Candidate genes may encode enzymes catalyzing biosynthetic steps in the production of compounds of interest, downstream catabolic processes, components of competing pathways, participants in compound storage or stability, regulatory genes and, possibly of most interest, genes representing unanticipated effectors of compound accumulation.

Obviously, we are at an early stage of mining this resource that may shed light on melon fruit biology by linking genomic and metabolomic data, providing useful markers for marker assisted breeding. The success of the project has already found its expression in initiating several new research projects using similar methodologies.

"Novel candidate genes for additional fruit quality traits of importance were identified"

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The Biochar Effect: Simultaneous Plant Growth Promotion and Protection from Disease

By Ellen R. Graber, Department of Soil Chemistry, Plant Nutrition & Microbiology, Soil, Water & Environmental Sciences Institute

About the first author

Dr. Ellen R. Graber is a principal scientist at the ARO. She began her research efforts into biochar in 2009, and established the Israel Biochar Research Network (iBRN) shortly thereafter. She serves as vice-chair of the COST Action TD1107 'Biochar as an option for sustainable resource management. Dr. Graber is engaged predominantly in multi-faceted and cross-disciplinary in agriculture and the environment. Her major current research activities are directed towards biochar use in agriculture, and involve understanding the mechanisms responsible for plant growth promotion and improved plant resistance to disease under biochar addition, as well as biochar chemistry and interactions between biochar and soil-applied agrochemicals and soil contaminants. Dr Graber was named Volcani Center "Scientist of the Year" for 2013, in recognition of her biochar-related research.

The problem of climate change has led to the search for new technologies to mitigate increasing levels of atmospheric greenhouse gases. One technology is **pyrolysis**, which converts waste biomass to gaseous and liquid biofuels and a solid carbon-rich product, **biochar**. Biochar is added to soils to improve their fertility. It doesn't easily breakdown, and therefore, fixes carbon that originated in the atmosphere in the soil for long times. Thus, pyrolysis of waste for energy and biochar production can be a ***carbon-negative*** process. We discovered that adding biochar to soil can simultaneously promote the growth of plants and activate their defenses against pathogen attack.

The problems of climate change (flooding, desertification, extreme weather events) have led to the search for new technologies to mitigate increasing levels of greenhouse gases in the atmosphere. One proposed solution includes reducing greenhouse emissions from biomass decomposition by carbonizing the biomass using a pyrogenic process. **Pyrolysis**, the thermal decomposition of biomass in the absence of oxygen, results in the formation of energy-rich products: liquid (bio-oil) and gaseous (syngas) biofuels, and a solid carbon-rich product, **biochar**.

While biochar can also be used for energy, the pyrolysis/biochar platform involves using the biochar as a soil amendment. Biochar is being widely studied around the world for its potential to enhance soil quality and promote crop growth.

The half-life of biochar in soil is hundreds to thousands of years or more, which means that adding biochar to soil can lead to very long-term carbon sequestration. If pyrolysis is done using modern equipment designed to trap and use the energy-rich gases produced during the anaerobic thermal decomposition of the biomass, rather than letting them escape into the atmosphere causing air pollution, pyrolysis of waste biomass for energy and biochar production can be a ***carbon-negative*** process (withdraws carbon dioxide from the atmosphere). Much agricultural waste and other kinds of waste streams (including forestry, food production wastes, manure, sludge, and municipal wastes) can be turned into biochar, bio-oil, and syngas via pyrolysis.

"……..biochar primes the plant's immune system"

In addition to its potential for carbon sequestration and decreased greenhouse gas emissions (being that wastes are converted into recalcitrant biochar carbon rather than decomposing to carbon dioxide), biochar has been reported to have numerous benefits as a soil conditioner: increased plant growth, reduced leaching of nutrients, reduced soil acidity, increased soil water retention, and reduced irrigation and fertilizer requirements.

However, this "Biochar Vision", which has attracted world-wide attention from many scientists and entrepreneurs, is still in its infancy. There are major research needs in the pyrolysis/biochar field, including but not limited to: (i) elucidating the mechanisms by which biochar influences soil fertility and crop yields; (ii) determining optimum application rates of biochar; (iii) evaluating the value of biochar in intensive, extensive and organic agriculture; (iv) quantifying the carbon sequestration and energy production potential of pyrolysis and biochar; and (v) finding inexpensive and efficient pyrolysis engineering solutions. Moreover, since biochar remains for such a long time in the soil, it is imperative to have a good understanding of how it may affect the soil and its productivity over long time periods before advocating its widespread use. It is also necessary to characterize and quantify possible detrimental effects of biochar in soil.

Our research program involves a multi-faceted approach aimed at providing insights to these questions and more, and involves intense research efforts to understand what happens in the complex soil-root zone-plant-water environment when biochar is added.

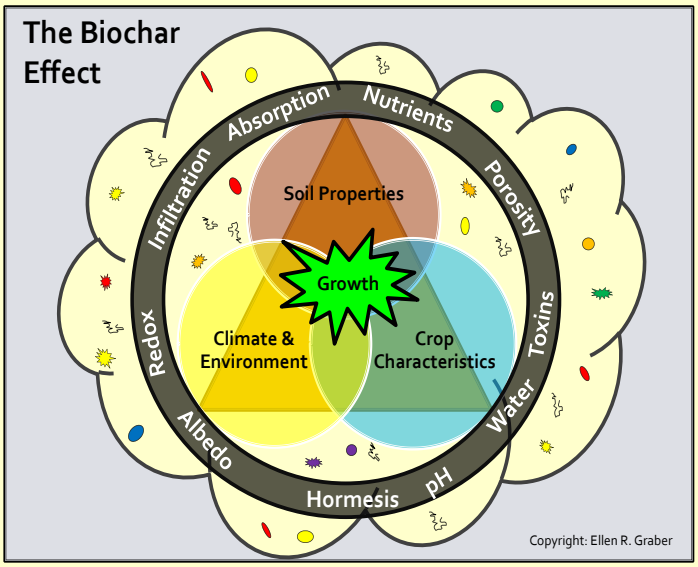
The Biochar Effect: Simultaneous Plant Growth Promotion and Plant Protection from Disease

In the first work of its kind[1], we discovered that a small amount of biochar in the potting medium served to reduce the severity of diseases caused by different fungal pathogens infecting the foliage of tomato and pepper plants. Pepper plants were also protected against damages from the broad mite pest. At the same time, the biochar-amended plants developed faster, despite the fact that all the plants, including those without added biochar, were optimally fertilized and watered. We termed this simultaneous plant growth promotion and induction of plant system-wide defenses "The Biochar Effect"[2], and have dedicated a number of our efforts to understanding it.

The fact that the biochar location (in the soil) was spatially separated from the site of infection (leaves) indicated that direct toxicity towards the disease-causing agents was not involved, and pointed to an induced systemic response of the plant to the biotic stress. Confirming this in studies with strawberry plants and foliar fungal pathogens having a variety of infection strategies[3], we revealed that the presence of biochar in the soil elicited the expression of defense-related genes in the plant along different metabolic pathways. We also found that biochar "primes" the plant's immune system, in other words, prepares the plant to activate its defenses more quickly than it would do if not primed upon pathogen attack. Our results are suggestive that the Biochar Effect and priming are the result of biochar-induced changes in the community of microorganisms[4] that develop in the root zone.

We have also studied the impact of different biochars on the development of diseases caused by pathogens that reside in the soil[5] in several different crop-pathogen systems. In contrast to systems involving diseases caused by above-ground pathogens, the placement of biochar in the soil environment means it can have both direct and indirect effects on the soil-borne pathogen, and hence on the development of diseases caused by those pathogens. We found that disease severity exhibited a U-shaped curve of response versus biochar dosage, with a minimum in disease severity at an intermediate biochar dose, and greater disease severity at lower and higher biochar doses. Importantly, plant growth also follows a U-shaped biochar dose/response curve, but usually the most effective doses for plant growth and for disease resistance are different. This means that a biochar dose which improves plant performance in healthy systems may result in suboptimal performance in systems under attack by pathogens. Thus, the importance of understanding the mechanisms responsible for various plant responses to biochar addition cannot be over-stated, particularly considering the longevity of biochar in soil.

".....this simultaneous plant growth promotion and induction of plant system-wide defenses "The Biochar Effect"



This figure presents our conceptual model for the interplay between the major factors affecting plant growth (labeled at the vertices of the triangle), biochar (represented by the dark gray ring), and microorganisms in the root zone (represented by the cloud). In order for a plant to grow and thrive, the three factors of the growth triangle (climate and environment, soil properties, and crop characteristics) need to be optimal. These factors can all be influenced by the root zone microorganisms, depicted as a cloud with a variety of microbes. The ring represents the effects of biochar on this system, where various physical and chemical impacts are labeled. The biochar-related aspects (e.g., adsorption, soil physical properties, toxins, redox, hormesis, nutrient supply and availability) can all influence different factors of the growth triangle both directly (depicted as the ring impinging on the vertices of the growth triangle), and indirectly through impacts on the root zone microbial community (depicted as the ring impinging on the cloud). Copyright: Ellen R. Graber.

Our studies suggest that changes in the severity of diseases caused by plant pathogens as a result of introducing biochar to the soil environment are related to a complex interplay of factors affecting the community of microorganisms in the root zone, the susceptibility of the host plant, the environmental conditions, and the virulence of the pathogen [6, 7]. Yet, given the paucity of data and the plethora of possible mechanisms, more research is clearly warranted. To date, only a handful of biochars and plant-pathogen systems have been tested, and most of the tested disease organisms have been fungi or related organisms. It is not known if biochar additions can impact diseases caused by bacteria, viruses, nematodes, and other soil organisms. Furthermore, it is not known whether the biochar effect will be protective in field situations over a number of seasons. This need is particularly acute considering that plant growth may be less sensitive to biochar dose in the absence of disease-causing pathogens than in its presence.

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Strigolactones, the new plant hormones, affect auxin flux by imposing changes in cytoskeleton & vesicle trafficking

By Hinanit Koltai & Nirali Pandya-Kumar, Department of Ornamental Horticulture, Plant Sciences Institute

About the first author

Dr. Hinanit Koltai is a senior research scientist in the Department of Ornamental Horticulture of the Plant Sciences Institute. She is involved in *in-depth* research of basic mechanisms of plant development aimed at the advancement of finding solutions for agricultural problems, both in the short and in the long term. Her recent breakthrough studies are in the field of strigolactones where she has demonstrated significant discoveries using an integrative methodological approach.

Strigolactones (SLs) are plant hormones that affect the development and architecture of different parts of the plant. SLs are sensed by plants via a specific intracellular and cytoplasmatic reception system. We found that SL-signaling triggers changes in F-actin and endosomal trafficking. As a result, they regulate the level of the auxin transporters PIN-FORMED (PIN) proteins in the plasma membrane (PM) of cells, and thereby, possibly, auxin efflux. The SLs-associated changes in F-actin and PIN protein localization may lead to either enhancement or depletion of PIN proteins from the PM and may underlie SLs non-cell autonomous effect.

Strigolactones-the new plant hormones

Strigolactones (SLs) are recognized now as plant hormones that affect the development and architecture of different parts within the plant. As such, they have become a focus of interest in plant science research. SLs were firstly identified back in 1966, as highly active germination stimulant of parasitic plant seeds isolated from cotton-root exudates[5]. The structure of the SL strigol was elucidated in 1972[6]. A first indication for the presence of SLs as plant hormones came from grafting studies in pea and Arabidopsis of a class of hyperbranching mutants.

The phenotype of these mutants could not be attributed to altered levels of one of the established plant hormones, such as auxin or cytokinin. These studies indicated on the presence of a novel, unnamed, "graft-transmissible signal" that suppresses shoot branching[3, 37]. This novel signal was only later defined as SL[12, 39]. Today, much more is known about SL activity (see also Box 1). They act to repress axillary buds outgrowth in shoot, by affecting locally in the bud and/or by affecting polar auxin transport[7, 9].

BOX 1- **Strigolactones act in plant interactions**

Strigolactones are produced and exuded from the roots and act as stimulators of seed germination in parasitic plants, including *Striga* and *Orobanche*[42]. SLs have also been found to play a role in other plant interactions—stimulating hyphal branching in the symbiotic arbuscular mycorrhizal fungi[42] and promoting the symbiotic interaction of plants with *Rhizobium*[10]. They are produced from a carotenoid precursor[24] mainly in the roots, in a wide variety of plant species, including dicots, monocots and primitive plants[42]. Their biosynthesis pathway includes activity of several enzymes[4], and their chemical structure consists of four-cycle skeleton of an ABC-ring system; the A and B cycles bear various substituents[44]. Today, a variety of SL analogs with biological activity are available[44].

Among strigolactones other activities in plants are positive regulation of shoot secondary growth[1] and negative regulation of adventitious-root formation[33]. Our group was one of the first two groups worldwide to find and characterize a role for SLs in roots. We and others have found they regulate lateral-root formation in association with growth conditions—suppressing it under sufficient phosphate, but inducing it under limiting phosphate[19, 34]. SLs were also found to increase cell numbers in the primary-root meristem[34, 22], and increase root-hair elongation in the primary root[19].

Moreover, we have found SLs to act as regulators of plant response to conditions of phosphate deficiency[25]. These finding suggest that SLs are important regulators of plant response to nutrient stress conditions, and may have further implications in agriculture.

BOX 2 - **Stigolactones reception system**

SLs are sensed by plants via a specific, intracellular and cytoplasmatic reception system[40]. This reception system consists of several components. One is an F-box protein, MAX2/D3/RMS4[14, 16, 37], which is linked to an Skp, Cullin, F-box (SCF)-containing complex[26]. D14, a member of the α/β-hydrolase fold superfamily[2] is the SL receptor and functions as a cleavage enzyme for SLs [13, 18, 28]. Furthermore, it was shown in rice that SLs induce proteasomal degradation of D53, a class I Clp ATPase protein in a D14- and D3-dependent manner and thereby prevent D53 activity in promoting axillary-bud outgrowth[15, 43].

"Strigolactones are important regulators of plant response to nutrient stress conditions ...."

Strigolactones are regulators of auxin flux

Despite the fact that the SL reception system (Box 2) is intracellular, several studies have suggested its effects to be non-cell autonomous[22, 33]. For example, in roots, we found that expression of *MAX2* under the *SCARECROW* (*SCR*) promoter in the SL insensitive *max2* mutants led to root-hair elongation in response to GR24[22]. *SCR* is expressed mainly in the root endodermis and quiescent center[35], suggesting that endodermal SL perception is sufficient so as to confer epidermal root hair elongation.

Perhaps the non-cell autonomous activity of SLs is derived from the fact that SLs are regulators of auxin flux. They dampen auxin transport in the shoot and thereby increase competition between axillary branches[7, 8]. In line with these findings, in the shoot, SL signaling triggers depletion of the auxin transporter PIN-FORMED1 (PIN1) from the plasma membrane (PM) of xylem parenchyma cells[36]. These findings support the hypothesis that SLs regulate shoot branching by modulating auxin transport[36]. In the root as well, several studies have suggested that SLs affect auxin efflux[21, 34] and PIN1 level.

"Strigolactones regulate endosome trafficking by affecting actin-filament organization ...."

Strigolactones alter PIN endocytosis and F-actin bundling

An additional insight into the activity of SLs in roots came from our study[30]. In this study we have shown that SLs increased PIN2 polarity in the epidermal-cell PM of the root. This was apparent in wild-type (WT) Arabidopsis but not in the SL-insensitive mutant *max2-1*. The PM localization of PIN proteins is largely dependent on dynamic vesicle cycling between the PM and the endosomes[11]. Indeed, SLs increased both PIN2 endocytosis and trafficking of the endosomal marker ARA7[38], suggesting that SLs may affect vesicle trafficking associated with PIN protein PM localization[30] (Figure 1). In addition, SLs were found to increase, to some extent, PIN2 transcription in the WT (but not in *max2*). Thus, SLs positively affect both PIN2 transcription and trafficking under the examined conditions[30].

Furthermore, we have shown in this study that SLs, in a MAX2 dependent way, decreased F-actin bundling and increased their dynamics[30]. F-actin is a major determinant of vesicle trafficking in the root epidermal and cortical cells, including vesicles that are involved with PIN2 recycling[11, 20, 23, 27]. Stabilization of actin filaments slows their dynamics and that of PIN trafficking and F-actin determines, to a large extent, the PM localization of PIN proteins[11]. Therefore, SLs, by affecting actin-filament organization and dynamics, may regulate endosome trafficking and PIN2 localization in the PM of the epidermal cell layer[30] (Figure 2).

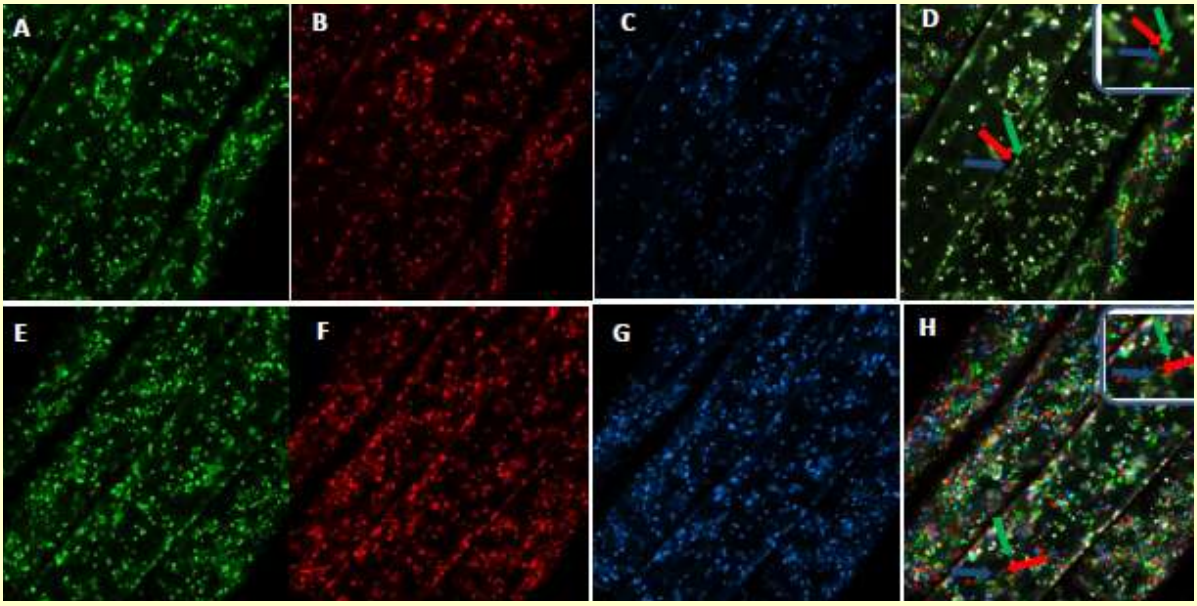

Figure 1 *Effect of GR24 (3 x 10^{-6} M) treatments on the velocity of ARA7-labeled endosomes in the epidermal cells of the primary-root elongation zone in wild-type seedlings. ARA7 labeled endosomes in seedlings that grew for 72 h on control (acetone)* ***(A-D)*** *or GR24* ***(E-H)*** *plates (scale bars = 10 µm).* ***(A, E)*** *Time frame in 0 sec (frame 1, green)* ***(B, F)*** *Time frame in 16 s (frame 5, red),* ***(C, G)*** *Time frame in 29 s (frame 9, blue),* ***(D, H)*** *Overlay of frame 1 on frame 5 on frame 9. Arrows in insert point to individual endosomes at Frame 1 (green), frame 5 (red) and frame 9 (blue).*

The direction of auxin flux is largely determined by the polar position of PINs in the PM[41]. Therefore, the increase in PM polarization of PIN2 inferred by SLs may result in increased auxin efflux in these cells. Moreover, elongation of the root-hair tip is affected by auxin transport within the epidermal cell layer of the root elongation zone[17]. Hence, the positive effect by SLs on actin-filament dynamics, endosome trafficking and PIN2 polarization in the PM of the epidermal cell layer may promote auxin flux towards root hair elongation zone and thereby, root hair elongation (Figure 2). Indeed, under the experiment conditions examined by us[30] SLs lead to increase in root hair elongation.

To summarize, studies from our laboratories indicated that the non-cell autonomous effect of SLs may be derived from their ability to trigger changes in F-actin, endosomal trafficking and PIN proteins polarity in the PM of cells, resulting with regulation of auxin efflux. Under different growth conditions or in different plant tissues SLs signaling may either decrease or increase PIN protein levels in the PM[30, 36]. It might be that SLs act on either "PIN removal parameter" or "PIN insertion parameter"[32]. Thus, SLs may act as regulators of the PIN-related auxin transport for either its enhancement or suppression dependent on growth conditions or tissue type.

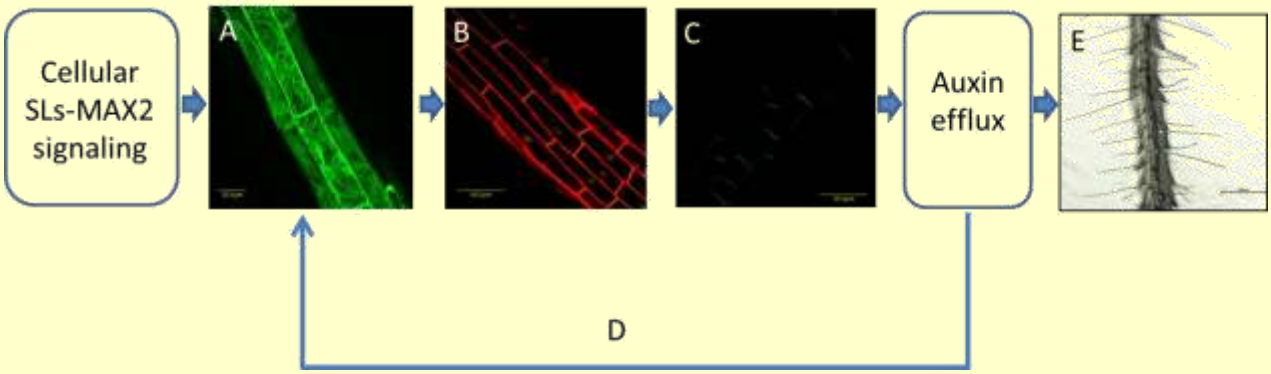

Figure 2 The effect of strigolactones signaling on root hair elongation is associated with changes in F-actin and PIN2 localization. *SL signaling, in a MAX2 dependent way, decreases the bundling of F-actin filaments (A). As a result, their dynamics is increase. The increase in F-actin dynamics increases PIN2 endocytosis (B) and the polarization of PIN2 in the plasma membrane (C). The increase in PIN2 plasma membrane polarization may result with increased auxin flux in these cells (D). Auxin by itself promotes its own transport (e.g., Nick et al., 2009D). These events probably result with increase in auxin flux towards root hair cells and root hair elongation (E).*

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Advanced edible coatings to improve quality and to prolong shelf life of fresh agricultural products

By Elena Poverenov, Department of Food Quality & Safety, Postharvest and Food Sciences Institute

About the first author

Dr. Elena Poverenov received her PhD in the Organic Chemistry Department, at the Weizmann Institute of Science in 2009 under the supervision of Prof. David Milstein. She carried out postdoctoral research in organic polymers at the Weizmann Institute of Science with Prof. Michael Bendikov. In 2011 she joined as a researcher at the Agricultural Research Organization, The Volcani Center. Her research group develops new food contacting materials that may improve quality and safety of agricultural food products. Her research interests are focused on biodegradable polymers as raw materials for advanced packaging, active edible coatings and encapsulation systems for active components. She employs nanotechnologies for protection, delivery or controlled release of active components. In addition, she also utilizes electrochemistry to tune and control properties of natural polymers.

Abstract

Edible coatings are a new promising approach for protecting food products from physiological and microbial deterioration. Coatings are based on natural edible polymers and respond to consumer demand for safe and environmentally friendly approaches of product quality control. To be applicable for fresh agricultural products, edible coatings have to answer a long list of various requirements. Our research group is developing composite edible coatings that combine the advantages of various natural materials and can benefit these multiple requirements. Two different approaches, blending and Layer by Layer (LbL) deposition are utilized to form advanced edible coatings. All the polymers we use are from natural sources and we utilize water as a solvent. We do not use organic solvents or synthetic additives. The method is cheap, simple and can improve the quality and safety of food products. The performance of the blended and LbL composite edible coatings was demonstrated on different fresh agricultural products. A blended chitosan-gelatin coating prolonged shelf life of red bell peppers from 14 to 21 days at cold storage and from 7 to 14 days at shelf-life storage. The alginate-chitosan LbL coating significantly prolonged shelf life of fresh-cut melons (up to 14 days vs the usual 5-7 days of storage) by slowing down tissue degradation and reducing bacterial and fungal growth. Carboxymethyl cellulose-chitosan LbL coating was found to considerably improve postharvest quality of 'Or' and 'Mor' mandarins, 'Navel' orange and 'Star Ruby' grapefruit. In all citrus species, the LbL edible coating provided fruit with shine, firmness, good flavor and prolonged storage-life.

Introduction

Environmental and microbiological damages cause deterioration of agricultural food products. Heat, oxidation, and fungal and bacterial attacks brought on by humidity impair the quality and the storability of agricultural products resulting in wastages that are detrimental to both farmers and consumers.

Edible coatings are a new promising approach for controlling the quality and extending the shelf-life of fresh agricultural products. Edible coatings may protect products from mechanical and microbial damage, provide an aesthetic appearance, and prevent the escape of favorable volatiles. Edible coatings are based on completely biodegradable and biocompatible polymers such as natural polysaccharides, proteins and lipids. Therefore they respond to consumer demand for safe and healthy food. Edible coatings satisfy environmental concerns and in many cases may provide an alternative to synthetic packaging and antimicrobial additives.

To be applicable on fresh agricultural products, edible coatings have to answer a long list of various requirements including (1) good adhesion to a product surface, (2) normal gas permeability (3) absence of color and flavor, (4) ability to improve the appearance of agricultural products, (5) ability to retard the moisture and the loss of favorable volatiles, (6) ability to maintain product firmness and textural integrity and (7) ability to reduce bacterial and fungal attack. Single coating materials are often unable to satisfy all these requirements.

Our research group is developing composite edible coatings that combine the advantages of various natural materials. Rationally designed multicomponent coatings could help to satisfy the diverse practical requirements. Two different approaches, "Blending" and "Layer by Layer (LbL) deposition" are utilized to form advanced edible coatings. All the polymers we use are from natural sources and water is utilized as a solvent, the use of organic solvents or synthetic additives is avoided.

Results

Blending is one of the most straightforward approaches for preparing multicomponent coatings. However, it has several limitations since the properties of the final coating material are only partially controlled (Figure 1).

The LbL method is an advanced approach based on the alternate deposition of various polymers to produce ultrathin layers on the surface. Physicochemical properties of the created films can be controlled by changing the deposition conditions and by altering the nature of the polymers themselves. This technique has an application range from optical devices to biomaterials. Despite the great potential of the LbL approach to control properties and its functionality as an edible coating, it has remained an almost unexplored method in the field of food quality and shelf life extension until recently. In our research, we utilize a combination of oppositely charged natural polymers, natural polymers that lead to a formation of an edible coatings of good quality (Figure 1).

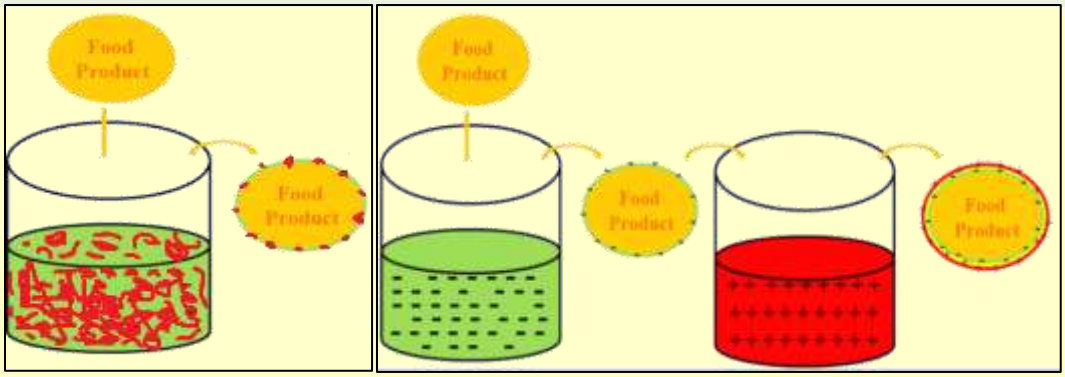

***Figure 1 Composite edible coatings**: Blending (left) & Layer by Layer methods (right).*

In our research we mainly utilize polysaccharides as raw materials for edible coatings. Polysaccharides have many advantages: they are low-cost, biodegradable, water soluble and safe. In addition, polysaccharides have well defined chemical structures that allow the tuning of their coating properties. For instance, cellulose is the most abundant natural polysaccharide that can be easily modified to produce a high variety of cellulose derivatives with variable properties. An additional polysaccharide of interest is chitosan, a de-acetylated form of chitin, which possesses intrinsic antimicrobial activity. Being a cationic polyelectrolyte, chitosan can be utilized in LbL that is rationally designed as edible coatings. Another interesting polysaccharide is alginate; a nutritional fiber produced from brown algae. In our research we also utilize gelatin, a natural protein widely used in the food industry.

The performance of the blended and LbL composite edible coatings was demonstrated on different fresh agricultural products. Blended chitosan-gelatin coating prolonged shelf life of red bell peppers from 10-14 to 21 days at cold storage and from 5-7 to 14 days at shelf-life storage.[1] After long shelf-life storage combined coatings effectively inhibited texture degradation and prevented shrinkage. The fruits were also artificially inoculated with *Botrytis cinerea*, the pepper damaging fungus.

The infection diameter of peppers coated by combined coatings was found to be significantly lower than that of the uncoated peppers or peppers coated by single layer coating (Figure 2).

"Edible coatings satisfy environmental concerns and in many cases may provide an alternative to synthetic packaging and antimicrobial additives.....

Rationally designed multi-component coatings could help to satisfy the diverse practical requirements ".

Figure 2 *Red bell peppers (top row) after 21 days of storage at 7°C and 5 days at 20°C compared to artificially inoculated with B. cinerea after 12 days of storage at 7°C (bottom row)*

In another research project, polyanion alginate- and polycation chitosan-based LbL edible coatings were applied on fresh-cut melons.[2] The quality of melons coated by these LbL coatings was compared to the quality of melons coated by single layer alginate or chitosan coatings and to the non-coated control fruits. The LbL coating was found to possess beneficial properties of both components of the layers. The LbL coated melons provided antimicrobial protection through the chitosan layer and texture enhancement properties through the alginate layer (Figure 3). The LbL coating provided melons with good firmness and microbial safety and as a result with enhanced quality and shelf life extension.

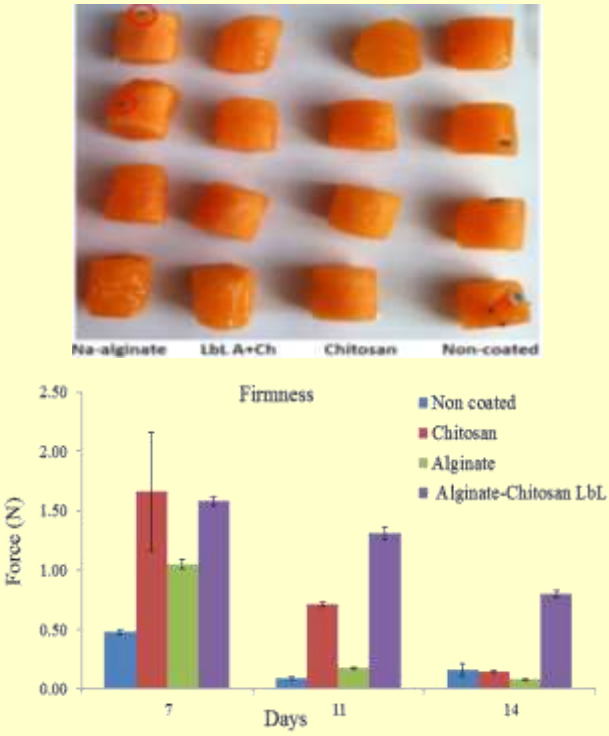

Figure 3 *Fresh-cut melons after 14 days of storage at 6°C (top). Firmness of fresh-cut melons during 14 days of storage at 6°C (bottom).*

In addition, we examined natural edible coatings for citrus fruit[3]. Currently, polyethylene based synthetic waxes are used for extending shelf life, protecting texture and improving the appearance of citrus fruit. The developments of coatings that are based on natural polymers that may demonstrate similar quality enhancement are very desirable. We have combined the good adhesion abilities of Carboxymethyl-cellulose (CMC) in the inner coating layer with the antimicrobial effect of chitosan in the external coating layer. The CMC-chitosan coating was applied on various fruits (Figure 4). Overall it was found that LbL edible coatings have improved performances and can be considered as applicative candidates for citrus fruit.

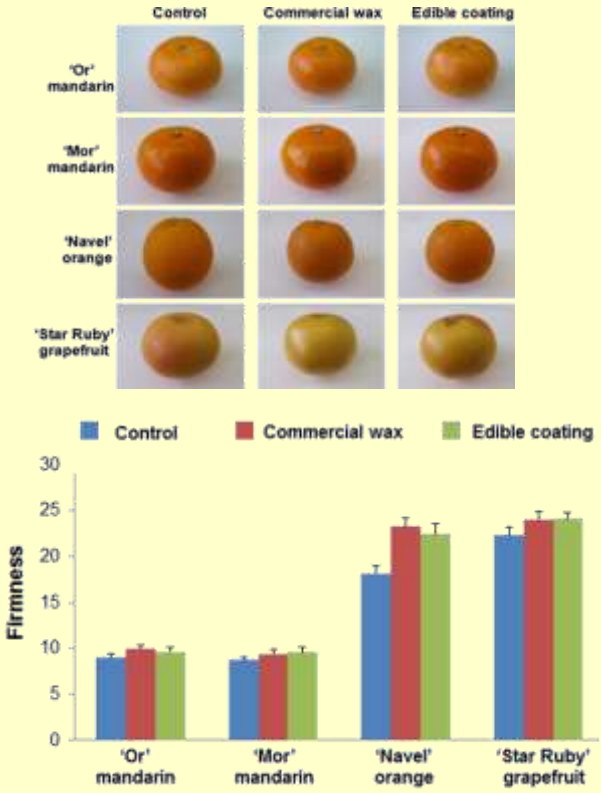

Figure 4 *Effect of commercial polyethylene wax and CMC-chitosan LbL edible coating on visual appearance (top) and firmness of citrus fruit (bottom). (Adapted from reference 1).*

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Implementation of thermal imaging for irrigation using ground mobile platform

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About the first author

Ronit Rud received her Ph.D. degree in 2011 in Agricultural Engineering at the Technion - Israel Institute of Technology. She is currently a research associate in the Institute of Agricultural Engineering at the Volcani center, Agricultural Research Organization (ARO). Her main interest is monitoring vegetation – crops, orchards and open areas – utilizing image spectroscopy and thermal imaging. Specific projects that she is involved in are: Characterization of salinity-induced effects in olive trees based on thermal imagery; Application of in situ crop water status mapping during the frowth season in vineyards and commercial potato fields; Use of environmentally sound and reliable techniques in Precision Agriculture (USER-PA); and irrigation management of commercial potatoe fields based on crop water stress index (CWSI).

Using ground mobile platform for thermal imaging places two main challenges: integration of the imaging system with the ground mobile platform and automatic extraction of thermal measures from images. During 2013-14 we conducted several imaging campaigns in commercial agricultural plots of crops and orchards from a ground moving platform. Various setups of imaging were tested including several heights, angles and speed. The operating interface of the imaging system was controlled by computer and was upgraded to include semi-automated thermal measures along with corresponding GPS points. This will enable integration of results into an outer database as a part of a spatial decision support system for management zones during the growth season.

Introduction

Thermal imaging is a known method for estimating the water status of plant. The images are used to map crop water status and can replace destructive measure of plants such as leaf water potential. Thermal imaging is more often adopted as a product of airborne platform. However, implementation of thermal imaging for irrigation, based on ground mobile platform may be more applicable in small management zones of agricultural plots.

The objective of this work was to enable spatial representation of thermal measure based on ground images from a mobile platform.

Materials and methods

Measurements site

Imaging campaigns were conducted in different climate zones and included various types of canopy structures from low density up to full closure in field crops and orchards (Table 1).

Table 1 Imaging site

Crop / Orchard	Plant annual cycle	Location
Grapes (*Merlot*), Israel	Ripening	31°52' N, 34°57' E
Apples (*Gala*), Switzerland	Fruit set – Fruitlet	46°23' N, 6°14' E
Cotton (*PF-15*), Israel	Beginning of flowering	31°46' N, 34°48' E
Potato (*Desiree*), Israel	Tuber initiation-bulking	31°28' N, 34°42' E

Imaging set up

Figure 1 depicts different mobile platforms, camera mounting and orientation. Camera height varied between 2.5 and 4.5 m from ground. Images were taken from a distance of 1 – 6 m. The imaging system integrated information from two cameras: a thermal infra-red (TIR), Flir (http://www.flir.com/cs/emea/en/view/?id=41415) and a regular Canon RGB and GPS. Imaging was performed remotely using a special software interface developed in the Institute of Agricultural Engineering.

Thermal data acquisition and processing

Imaging was conducted around the time of solar zenith (11.30 am-2.30 pm). Images were acquired at the highest speed along with the GPS points. Due to limitations of data transfer, synchronization and recording; the maximum speed of imaging was 2 seconds. Image processing comprises several stages: Separation of canopy from background, choosing pixels that represent the area of interest on the plant/tree; and finally, calculating canopy temperature and crop water stress index (CWSI)[3,4]. All processes were applied on data of the TIR images taking meteorological data into consideration[6].

The imaging system provided the basic spatial data of canopy temperature. By normalizing the canopy temperature with consideration of the meteorological conditions (affecting plant's transpiration and temperature) we can calculate the CWSI. CWSI values were in the range of 0-1, larger values indicating higher water deficit stress.

Plant sampling and analysis

An indication for plant water status is the plant water potential and stomatal conductance. These two parameters were used as reference measures and were determined by pressure bomb and porometer from sampled leaves, respectively. A detailed procedure for measurement of leaf (LWP) or stem water potential (SWP) and stomatal conductance in leaves of cotton, grapes and potato has been previously described [2, 4, 5, 6]. These well-known methods are accepted as plant measures of water stress and were used by us to verify image measures. Comparison and statistical test of correlations were conducted according to Alchanatis et al[1].

"…..*plant water potential and stomatal conductance were used as reference measures*."

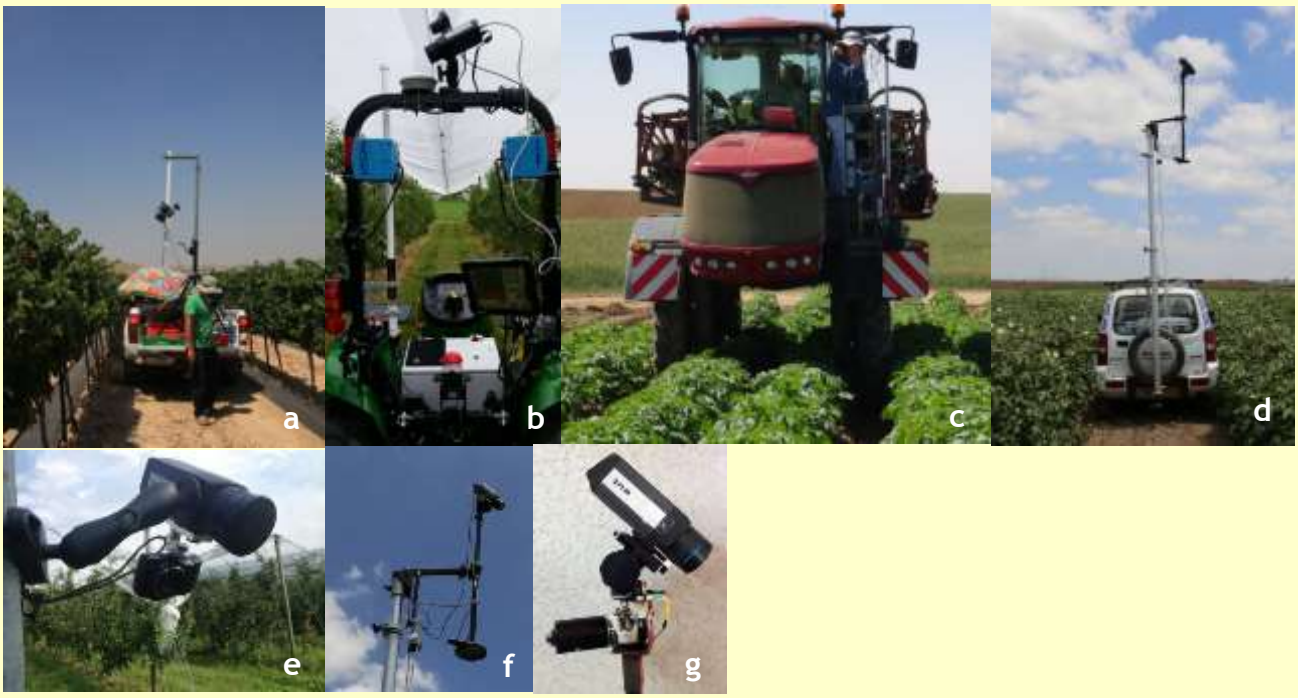

Figure 1 *Common field vehicles (a, d), autonomous small tractor (b), sprayer (c), fixed mounting to a bar (e), to a swinging bar (f) and rotating head remotely controlled (g).*

Meteorological data

Global radiation, wind speed, air temperature and relative humidity were measured by a mobile meteorological station. In case of Israeli measurement sites the station was positioned within the experimental plot, its sampling rate was every 10 sec (excluding wind which was measured every 1 sec), and 1 min averages were recorded by a data logger (Campbell Scientific, Logan, UT, USA). In case of measurement site in Switzerland it was measured by regional meteorological service, Agroscope (http://www.agrometeo.ch/fr/meteorology/datas). The station was about 1 km from the orchard and for each parameter 10 min averages were recorded. Meteorological measurements were performed throughout the data collection.

Results and Discussion

Table 2 shows different sampling rates while using the mobile moving platform.

In case of high-medium driving speed (5.5, 9 km/h) less sampling points can be collected, therefore this setting is suitable in a crop field or in a dense canopy with full foliage. The low speed (1.5 km/h) driving is suitable for working in an orchard since it gives more frequent sampling points. Moreover it enables the production of more accurate information of the canopy. Medium-high speed imaging results in a rather random sampling and less accurate canopy description. According to the desired accuracy and efficiency by the farmers the speed of the mobile platform can be optimized.

Table 2 Comparison of different sampling rates

Driving Speed [km/h]	Imaging rate [sec]	Distance between sampling points [m]
5.5	5	7.6
9	5	12.5
5.5	2	3
9	2	5
1.5	2	0.8

Field imaging from a mobile platform is preferable from an oblique view although the optimal imaging position would be perpendicular to the land surface. The advantage of oblique view is that a greater area can be scanned therefore the imaging can be accomplished in the time limitation around the solar zenith. Two positions of the cameras were tested; Figure 2a shows a scanning in 45° and Figure 2b in 0°. The imaging angles and distances from the canopy within the image field of view were different in the two setups, which resulted in differences in the number of sunlit pixels and variation in canopy temperature as well (Figure 2c, 2d).

The most important factor regarding this issue is the timing of imaging considering sun elevation and azimuth angles during the day. As a result of comparing the two setups, the oblique view imaging was chosen, giving lower standard deviation (July and August) and higher number of sunlit pixels. In conclusion it is strongly advised to scan the plots at the same sun elevation and same illumination angle whenever a time series of imaging is done.

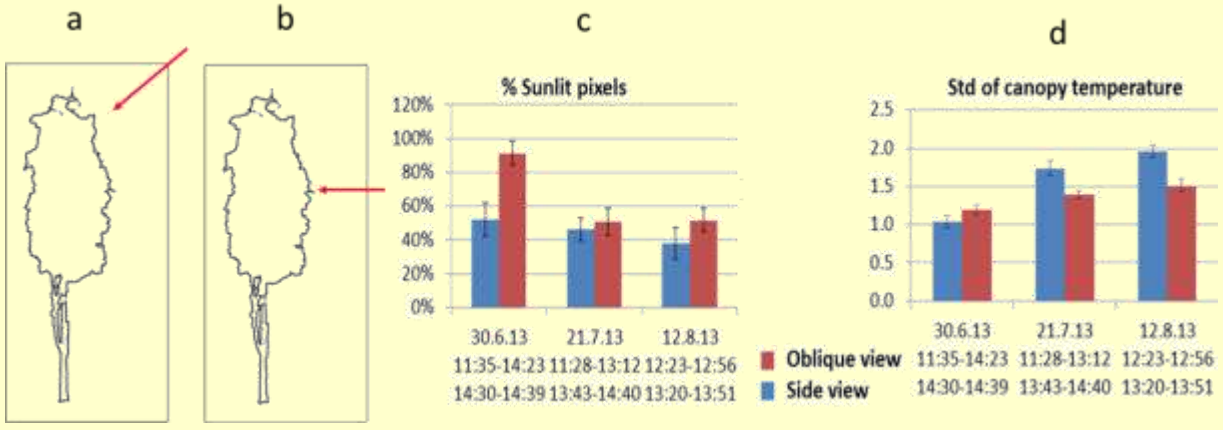

Figure 2 *Camera positions (a, b) and variation of measures within the images (c, d)*

Since the focus of this work is the representation of thermal measures produced by ground mobile platform, it includes the use of existing models which translate image measures (CWSI) into more operative values that famers can apply, such as LWP or SWP in grapes and cotton[5, 2]. For potato there are no common plant measure that is used for this purpose therefore we developed a preliminary correlation model[6]. Figure 3 shows different tools and concept of visualization of spatial thermal information. For the case when no correlation models exist, the thermal images (canopy temperature or CWSI) are used alone (Figure 3a) and when there are models, the thermal information is transformed and presented as plant measure (Figures 3b and 3c).

> "*Using such a setup may lead to the understanding of a constant problem visualized as an outstanding value and thereby can lead to a decision such as changing the irrigation lines.*"

The heat map (Figure 3a) shows variations in temperature of an orchard. It might be misleading considering irrigation decisions, since it shows temperature values without the consideration of weather conditions. In addition, it might be that an unusually high temperature relates to an open space or to a small tree (younger or pollinator tree which characterized with smaller leaves), therefore it indicates a suspicious point worthwhile special attention. The LWP map (Figure 3b) demonstrates integration of thermal image information with the irrigation system. There, each mean value relates to different irrigation sector. Figure 3c shows an image of value collection along a driving path as the mean value of small area (about 10^2 m). Using such a setup may lead to the understanding of a constant problem visualized as an outstanding value and thereby can lead to a decision such as changing the irrigation lines. This thermal line scan is more economical and practical. Moreover, it may save the amount of applied water by tuning it according to the need of the crop.

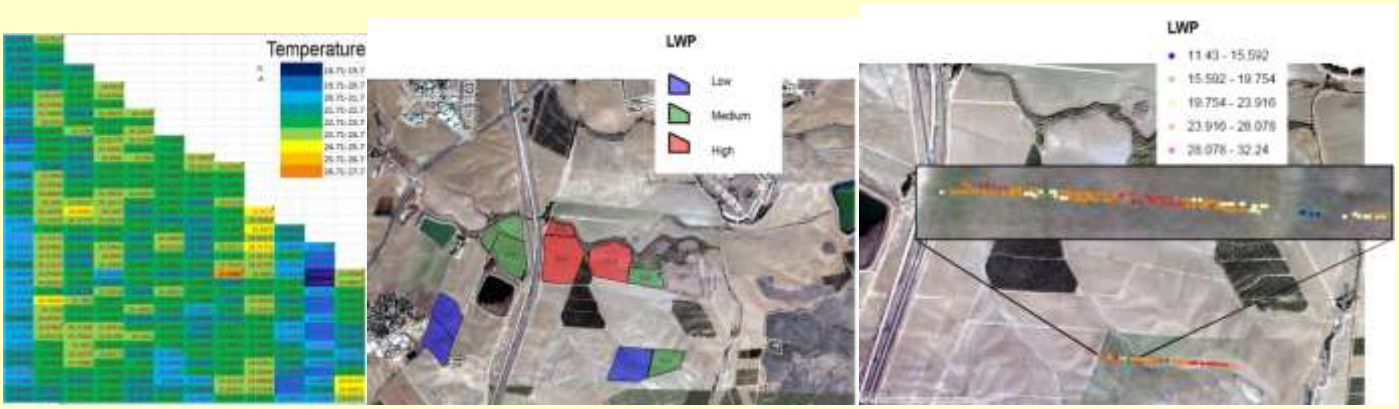

Figure 3 *Variation of temperature in apple orchard (a), mean LWP in irrigation plots of cotton (b), LWP point values in cotton (c)*

Conclusions

Medium and high speed thermal imaging using ground mobile platform was found to be suitable for agricultural plots with dense canopy where the image field of view is almost fully covered by the crop foliage. Low speed mobile platform was found to be suitable for orchards and less dense vegetation. The oblique view imaging was found to be preferable, giving lower standard deviation and higher number of sunlit pixels.

As thermal imaging is an adequate method of gathering information on water stress of plantations or crop fields, an economical solution which can be applicable to farmers in the small scale plots is needed. The thermal line scan with a correlation model and suited meteorological data can provide an economical and practical on-line solution for the farmer

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New approaches to control the honey-bee parasite,

Varroa destructor

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About the first author

Dr. Victoria Soroker did her Ph. D in insect physiology at Hebrew University University under the supervision of Profs. Ada Rafaeli and Rachel Galun, specializing in "neuroendocrine regulation of pheromone production in moth". Later she pursued her postdoctoral studies in the State University of New York at Stony Brook USA, specializing in pheromone and hormone binding proteins in moths under the supervision of Prof. Glen Prestwich. Subsequently she specialized in pheromonal communication of social insects and particularly ants and bees at Tel Aviv University under Prof. Abraham Hefetz. Victoria joined the ARO in 1999 and established a chemoecology lab studying chemical signals modifying arthropod behavior.

Varroa destructor Anderson & Trueman (Acari: Varroidae) is an obligatory ectoparasitic mite of honey bees *Apis mellifera* and *Apis cerana* (European and Asian honey bees respectively) and is one of the major threats to European honey bee colonies almost worldwide. Chemical signals from the bee are known to play a major role in host detection of *V. destructor*. Apparently, specific chemosensing of *V. destructor* allows it to distinguish between bees of different age and tasks. Our approach to provide control measures against the mite includes the interference in *Varroa*-host detection via the disruption of the host identification/orientation process by the mite. In search for a compound that specifically disrupts the interaction between the *V. destructor* and its host, we developed a method to study the chemosensing of *Varroa* using its main olfactory organ situated on the foreleg. Using the electrophysiological assay on isolated foreleg we screened putative chemosensory disruptive compounds. In particular, we examined the effect of volatile compounds originally developed for disruption of pheromone detection by moths and a commercially available repellent N,N-Diethyl-meta-toluamide DEET. The effect of disruptive compounds was further tested on host location by mites. Two types of electrophysiological effects were evaluated on isolated foreleg: short term inhibition and long term inhibition. Using this approach, several potent chemosensory disrupting compounds for the *Varroa* were identified, thus opening new avenues for the control of this major honeybee pest.

Introduction

Varroa destructor Anderson & Trueman (Acari: Varroidae) is an ectoparasitic mite of honey bees, originally found in the Eastern honey bee *Apis cerana*. A shift to the European honey bee *Apis mellifera*, which lacks the protective traits (eg. intensive hygenic behavior, luck of reproduction in worker brood cells) of its original host, has led to an unbalanced host-parasite relationship and to devastating damage to honey bee colonies. Today, the *V. destructor* has spread almost all over the world and is considered to be the most significant threat to apiculture, and as one of the major causes of the Colony Collapse Disorder[3]. The mite is harming honey bees in many ways both directly and indirectly. It feeds upon the hemolymph (blood) of the bee, leading to a reduced weight and life span of the emerging adult bee[3]. In addition it has been reported to cause neurological damage resulting in reduction in flight ability and non-associative learning and to weaken the immune system, increasing the bee's sensitivity to pathogens[3]. However, the main damage that leads eventually to the collapse of the hive is probably via the viruses that it transmits[1].

The mite first appeared in Israel in 1984 and since then has been the main threat to the local apiculture, requiring regular treatments, since *Varroa* reproduction in Israel continues all year around- *Varroa* control has been based on chemical pesticides and until recently for twelve years mainly on Coumaphos. This method crashed about two years ago. The loss of activity can be probably explained by evoked resistance in the *Varroa* population. A similar phenomenon was already observed in other countries. Moreover, there are two major problem concerning remedies against *Varroa*: 1. Both the *Varroa* and honeybees are evolutionary related, so pesticide efficient against *Varroa* usually also have negative effect on honeybees. 2. Residues of pesticides may get to bee products which are intensively used for human consumption, medicine and cosmetics. It is now understood that, like in other fields of agriculture, also in apiculture, in-hive pesticides are not the answer to sustainable honey bee health maintenance. Therefore, an integrated approach to *Varroa* control has been advocated. In spite of a world wide effort to solve the *Varroa* problem, -efficient remedies are not yet discovered. The objective of our study was to identify new potential *Varroa* control agents, particularly those disrupting bee recognition by the *Varroa* mite.

The Varroa life cycle is well synchronized with that of the honey bee and reproduces exclusively within the honey brood cells. As the synchronization between the Varroa's and the bee's life cycles appears to be the key for Varroa success, the disruption of the Varroa host finding behavior is a good approach for its management. Varroa do not have eyes or antennae, their host detection depends mainly on their sense of smell. The major olfactory organ of the Varroa responsible for sensing the bee volatiles is concentrated in a pit organ located on each of the front legs[2]. This unique organ contains sensory organs that consist of nine internal sensilla and nine long hair surrounding sensilla. Some of the sensilla (at least six) are wall pore sensilla that bear similarity to the olfactory sensilla of other arthropods (Figure 1A).

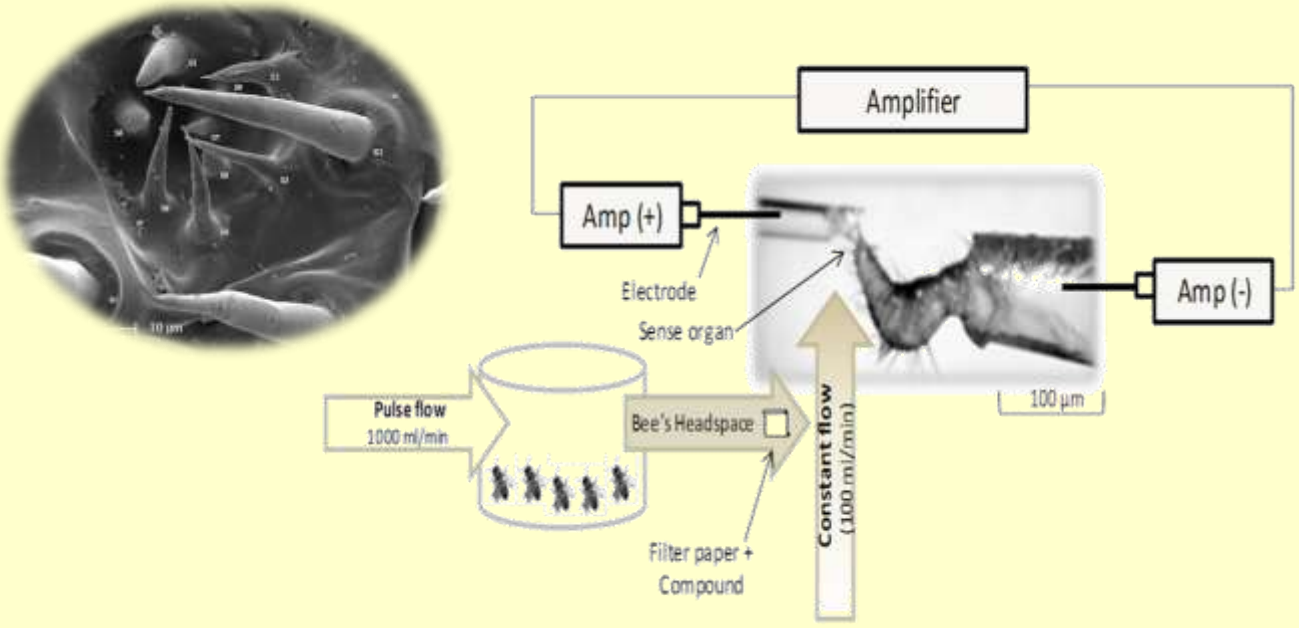

Figure 1 Scanning electron microscope (SEM) analysis and EAG set up for the isolated foreleg.(A) *SEM showed that isolated foreleg consist of sensilla (at least six) are wall pore sensilla.* ***(B)*** *The set of the EAG (schematic, not to scale), measuring Varroa foreleg electrophysiological response to a stimulus of Bees' headspace. A clean air flow is injected to a closed, environmental controlled jar, containing 5 frizzed-killed nurse bees. Subsequently, the same volume of bees' headspace is emitted from the jar, onto the Varroa foreleg. Any change in the electrical potential of the leg (depolarization) is amplified and recorded by the computer program (mV).*

Materials & Methods

Over twenty chemicals, most of which were originally developed in EP laboratory for disruption of sexual communication in Lepidoptera were tested along with commercial repellents such as N,N-Diethyl-meta-toluamide (DEET) were evaluated by electrophysiological (EAG) and behavioral bioassays. In this case the EAG assay was developed on the isolated foreleg of female *Varroa* mite (Figure 1A). The foreleg was stimulated by puffs of honeybee odor, or a clean air (control) as shown in (Figure 1B). The tested chemicals were blown with or without bee odor. The response amplitude was recorded for each stimulus.

The effect of compounds on the behavior of female *Varroa* was tested by a choice assay in a glass arena[2] (Figure 2). Briefly, in the presence of the chemical or solvent as a control, the mites were given a choice of two bees (a forager and a nurse). The *Varroa* host preference was assessed after 2 hours.

".....a promising solution for breaking the synchronization between the Varroa and the bee"

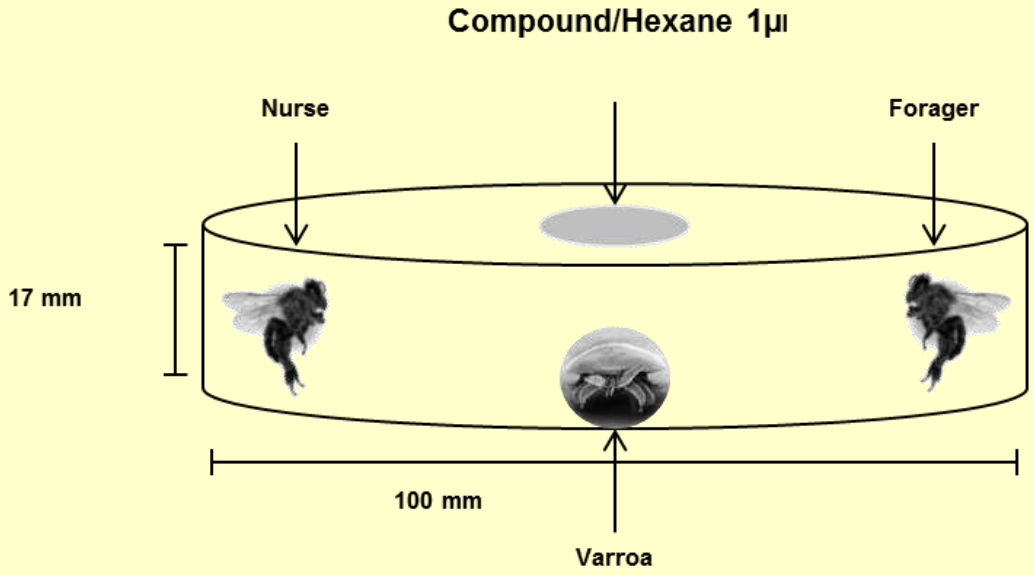

Figure 2 Experimental setup for the choice bioassay: The test compound did not contact the mite, and the mite could move around and choose between a freshly killed nurse or forager bee

Results and Discussion

In this study we – show for the first time not only that that the *Varroa* senses honeybee odor via its sensory organ in the pit organ, but also that that some of the tested compounds elicit disruptive effects on – *Varroa* behavior. The effects apparently start at the level of the sensory organ.

Two effects were detected by the EAG assay: 1) short-term inhibition-decreased responses to honey bee headspace volatiles when the compound was given simultaneously and 2) long-term inhibition – decreased responses to the following stimulation by honey bee headspace volatiles. The response type varied according to the disruptive compounds applied. Figure 3 present examples of responses acquired with 4 compounds:.– compound **3c**{*2,2*} caused only short term effect whereas compound **cy**{4*,1*}, **3b**{*2,2*} and DEET[4] showed a long term significant effect. Following EAG, choice bioassays were performed mainly with compounds that showed inhibitory effects.

As expected, the results clearly show that most of the mites prefer nurse bee over forager in the presence of control hexane (Figure 4). Whereas in the presence of compounds **cy**{4*,1*} and **3b**{*2,2*}, most of the mites' preference changed from nurses to forgers, whilst the total number of mites reaching foragers did not change. Interestingly, in the presence of DEET the preference did not change but the majority of the mites did not reach any of the hosts.

The mode of action of these compounds on the *Varroa* is still unknown, but currently under study in our laboratory. Moreover, not much information exists on the structure and function of olfactory machinery in mites. Theoretically, the EAG inhibiting compounds can interfere with any of the events prior to the activation of a sensory neuron: from the first stage, at which the inhibiting compound may include complex interaction within the odorant receptor neuron (ORN) machineryto the interference of the interaction of the bee odorant with its respective receptor in *Varroa* and the subsequent recovery of the system by the action of arrestin and ion pumps as reviewed by Plettner and Gries[5].

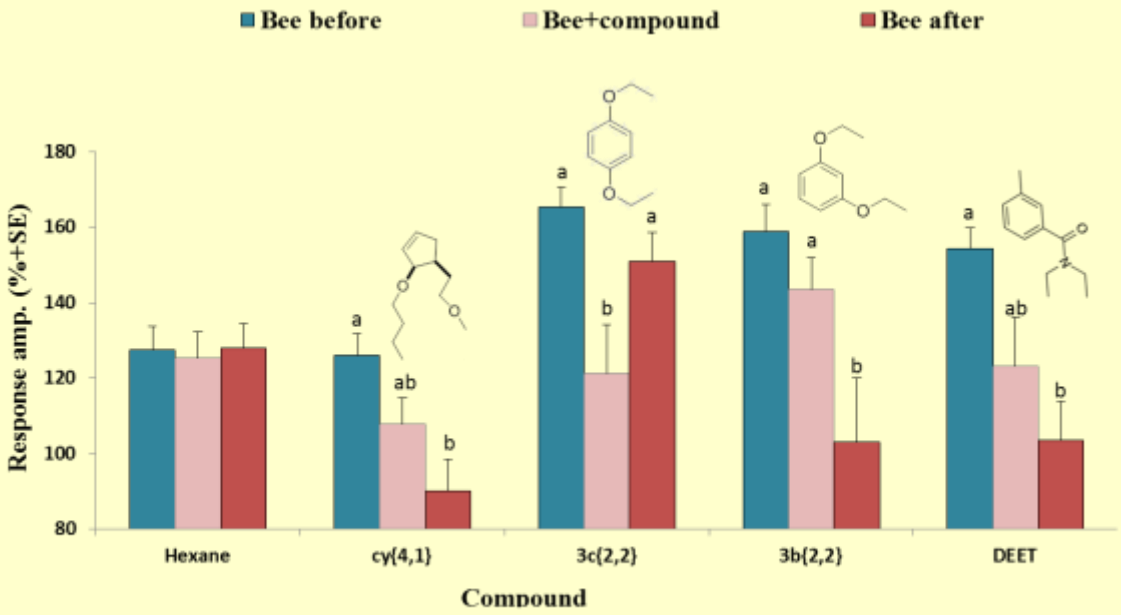

Figure 3 Electrophysiological responses for different compounds (10 µg) on Varroa foreleg. *The time interval between each stimulus was 30 s. The stimuli were: Air, Headspace of five nurse bees (Bee stimulus), Bee stimulus together with the compound (Bee stimulus + comp) or of the hexane control (Bee stimulus + hexane). For the bee stimuli, the headspace from 5 nurse bees was used (normalized values against the response to air %, average + SE). Anova repeated measures followed by Tukey–Kramer post hoc tests. Bars marked by different letters are significantly different, p < 0.05, (n = 6–7).*

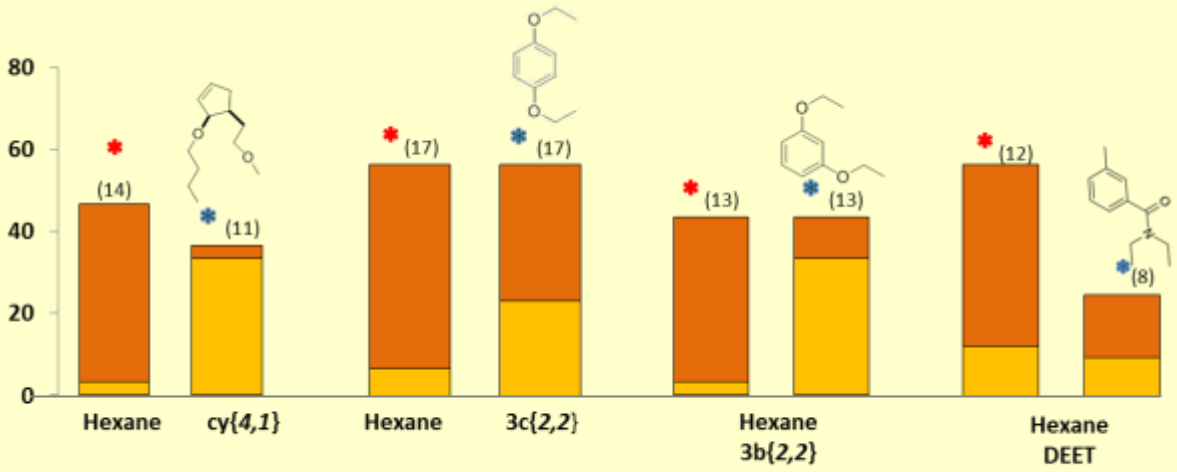

Figure 4 The effect of compounds on Varroa host choice between a nurse and a forager bee. *The effect of different compounds on Varroa host choice between nurse and forager bee. Each compound was tested in 10 µg versus Hexane, as a control. The numbers above the columns indicates the total number of Varroa choosing any of the hosts, 120 min from the beginning of the experiment. The data are percentage out of "n". A red star (*) above the column indicates that the mites significantly preferred a nurse over a forager bee; whereas a blues star (*) indicates a significant preference for a forager bee. Goodness of fit, p<0.05.*

In summary, the present study provides evidence of the ability of a number of synthetic compounds to disrupt host selection by *Varroa* mites by inhibiting its host sensing. These findings are breakthroughs for both basic science on mites chemosensing and apiculture. In apiculture, as the compounds are supposed to be implemented in a hive, their effect on honeybees and residues in bee products must be evaluated in the future. Although implementing communication disruption is still far from practice, it provides a promising solution for breaking the synchronization between the *Varroa* and the bee, thus promoting the ability of bees to survive infestations. This approach is a promising step towards an integrated and sustainable control over this major apicultural pest.

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".....tested compounds elicit disruptive effects on Varroa behavior."

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