Do you speak chemistry?

Small chemical compounds represent the evolutionary oldest form of communication between organisms

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In his famous fantasy epic, *The Lord of the Rings*, British writer and Professor of English Language and Literature, J. R. R. Tolkien, invented the Ents: tree-like creatures who were some of the oldest beings in his fantasy world of Middle-earth. In Tolkien's story, the Ents used a unique and ancient language to communicate with each other in a very deliberate way. Tolkien described this language as "unhasty" and "slow, sonorous, agglomerated, repetitive, indeed long-winded".

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Of course, Entish is a fantasy language, but the idea of a "language of trees" touches on the real-world question of whether plants actually do communicate. In 1983, Ian T. Baldwin and Jack C. Schultz answered this question in an article in *Science*: "Rapid Changes in Tree Leaf Chemistry Induced by Damage: Evidence for Communication Between Plants". In their paper, Baldwin and Schultz suggested that airborne signals originating from damaged plant tissues stimulate biochemical changes in adjacent plants. These changes in turn affect or deter herbivorous insects [1]. The publication inspired numerous articles, features, and comments in the press about "talking trees"—the intriguing idea of plants chatting with each other proved fascinating to the general public.

Nevertheless, although they do not chat in Entish, plants do communicate. In a very basic sense, and according to Shannon & Weaver's *The mathematical theory of*

communication (1963), communication involves a sender, a signal, and a receiver, the last of which is able to recognize and decode the signal. Plant communication definitely fits this model (Fig 1). However, communication between living organisms is actually much older than the 450 million years that land plants have lived on land. The oldest form of communication is probably the chemical interactions that occur between microorganisms and between microorganisms and their environment. This simple communication is based on the exchange of chemicals and probably evolved to improve the ability of microorganisms to find and acquire nutrients and mating partners, or to sense and evaluate environmental conditions [2]. Following on the heels of bacteria and simple eukaryotes, these chemical sensing and communication systems further evolved in plants, insects, and other higher organisms.

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Plants are able to recognize and respond to many different signals from their environment. These include physical signals from the abiotic environment—such as light, humidity, or temperature—and signals from the biotic environment—such as pathogenic and symbiotic microbes, or phytophagous organisms—all these signals are almost

always chemical compounds. In principal, these compounds are provided by a sending organism and specifically recognized by the receiving organism. If a particular chemical cue triggers signal cascades in the receiving plant that eventually lead to gene expression and appropriate physiological responses, the requirements for communication are fulfilled (Fig 1). It is clearly a matter of chemistry-based information and communication: a chemical language.

As primary producers, autotrophic plants are at the bottom of any food chain. Even long before the appearance of flowering plants, the earliest land plants were attacked by microbial pathogens and later on by herbivorous animals. Given their immobility, plants evolved both sophisticated recognition and defense strategies very early on in order to fend off the incessant attacks [3]. Plants can recognize microbial compounds in a way that allows them to identify the specific microbe attacking them—a prerequisite for a fast response. These compounds are called microbial-associated molecular pattern (MAMPs) and can bind to corresponding receptors on plant cells [4]. MAMPs are a variety of essential structures—ranging from polypeptides and peptidoglycans to carbohydrates, sterols, and lipids—and are therefore highly conserved within the realm of bacteria [4].

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Figure 1. Simplified model of communication between two organisms.

The information (green arrow) released by A cannot be used by the non-receiver B but only by receiver C that decodes the information (blue arrow) and responds adequately with C*.

Insects represent by far the largest animal group with respect to both number of species and individuals. The first insects evolved about 480 million years ago and probably co-colonized land at the same time as plants did [5]. It is therefore not too far-fetched to speculate that plants and insects probably interacted with each other from the beginning, even if the earliest fossil records of terrestrial herbivorous arthropods date back to about 420 million years ago. Over the millennia, plants have evolved a spectrum of defense strategies to drastically reduce insect feeding. Besides physical defenses—such as thorns or trichomes—plants constitutively synthesize a variety of toxic compounds that are stored in their tissues. These include glucosinolates, cyanogenic glucosides, terpenes, and alkaloids that can all kill insects and other animals feeding on the plant. Interestingly, humans are the only species that have been deliberately using many of these toxins—atropine, digitoxin, nicotine, morphine, artemisinin, and so on—for medical, cosmetic, or recreational purposes.

In addition to the constitutive production of defense chemicals and physical armaments, the impact of feeding on a plant induces many on-demand defense responses, most of which are chemically based [3,4]. The activation of the complex signaling network to establish such downstream responses requires the plant to recognize the presence of the aggressor quickly and efficiently [3]. To make it even more complicated, constitutive and inducible defense mechanisms can be further characterized as direct and indirect defense modes. Whereas a direct defense by toxins or repellents aims at affecting the survival or performance of the attacking organism, indirect defenses protect the plant by recruiting other organisms (Fig 2): Ants, for example, are attracted and rewarded with extrafloral nectar to attack herbivores. Even more

striking is the attraction of parasitoids, such as certain wasps or the specific predators of an attacking herbivore, which is achieved by releasing volatile organic compounds (VOCs) [3,4]. Thus, in addition to a first trophic level represented by the plant and a second trophic level represented by the herbivore, indirect defenses employ a third trophic level for defense. This strategy of indirect defense by attracting predators of herbivores is often called the “cry for help”.

The release of different bouquets of VOCs has been demonstrated for many different plant species. Chemically, VOCs belong to different groups: terpenoids, the so-called green leaf volatiles—fatty acid-derived C6 volatiles and derivatives, alkanes, alkenes, and alcohols—and some aromatic compounds—indole, methyl salicylate [3]. In principle, blends of VOCs can carry different types of information: information for the herbivores to localize the host; information for indirect defense by attracting the next trophic level; information for distant parts of the same plant; or information for neighboring plants to adjust their defensive status [1,3].

This last aspect was demonstrated by R. Karban and colleagues in field studies: Induced VOCs released from sagebrush (*Artemisia tridentata*) were able to trigger a higher resistance against herbivores in neighboring conspecific and wild tobacco (*Nicotiana attenuata*) plants, but not in three other forbs that grow in close vicinity to sagebrush [6]. This must mean that the chemical language of plants is not universal and that not all plants in the same habitat are able to get the same messages. They either cannot perceive or cannot decode the communicated signals. However, it is still a matter of debate—and a philosophical question as well—whether or not the VOC-releasing plants intend to alarm neighboring plants to prepare against herbivores that are around,

or whether the plants in the vicinity of the infested plants simply eavesdrop on the plant under attack and take advantage of the information that was released to the environment.

Chemical compounds similar to MAMPs have also been identified in herbivorous insects, and plants use these so-called HAMPs—herbivory-associated molecular patterns—as signals to recognize the herbivore [4]. Often, such HAMPs are in the feeding insect’s oral secretion, which consists of fluids from glands in the mouth part and the foregut, which is inhabited by microbial communities. Similar to MAMPs, HAMPs include structurally completely different compounds: Disulfoxy fatty acids (caeliferins) have been identified from the American bird grasshopper (*Schistocerca americana*), β -glucosidase and benzyl cyanide derivatives from cabbage white butterfly (*Pieris brassicae*), and the peptide inceptin from fall armyworm (*Spodoptera frugiperda*) [4].

The most prominent and fully characterized HAMP is volicitin (*N*-(17-hydroxylinolenoyl)-L-glutamine), which was isolated from beet armyworm (*Spodoptera exigua*) oral secretions [7] (Fig 3) and can be used as a model compound to study plant responses. For instance, the application of volicitin to damaged corn (*Zea mays*) seedlings induces the release of a certain blend of VOCs that attract parasitoid wasps, whereas mechanical damage alone does not trigger the same emission [7]. Meanwhile, various *N*-acyl acid amino acid conjugates have been isolated from the oral secretion of several lepidopteran larvae, crickets, and fruit fly larvae [4,8]. All these *N*-acyl acid amino acid conjugates represent derivatives of volicitin that can induce the release of VOCs in certain but not all plant species.

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An interesting finding is that at last fourteen genes that encode enzymes able to synthesize *N*-acyl amino acids have been

Figure 2. Model of plant–insect herbivore interactions and associated chemical defense mechanisms.

Feeding insects above- and belowground face direct chemical defense responses; some insects are able to detoxify the defensive compounds. Indirect defenses are represented by extrafloral nectar that can attract ants to defend the plant or by the emission of volatile organic compounds. These volatiles attract enemies of the herbivores, that is, organisms of the third trophic level (predators, parasitoids). The figure was kindly provided by Dr. A. Burse and further modified.

***N*-acyl-glutamines (*Spodoptera exigua*, Lepidoptera)**

Figure 3. Structure of the best characterized herbivore-associated molecular pattern.

[*N*-(17-hydroxylinolenoyl)-L-glutamine] was isolated from oral secretions of *Spodoptera exigua* larvae. Derivatives were found in many other insects.

The obvious contribution of bacteria to the biosynthesis of HAMPs that play a crucial role in the interaction of plants, herbivores, and their predators implies the existence of an additional trophic level to this network—the role of which is currently underestimated—and the coevolution of plant–herbivore interactions. Also, assuming that interactions between plants and microbes are even older than interactions between plants and insects, the bacterial origin of these particular HAMPs suggests that plants are simply using ancient and well-known chemical signals in a new context to realize that a herbivore is attacking them and mount a defense. Why developing new features when old ones are still efficient?

These chemical interactions between plants and insect herbivores or pathogenic microbes are subject to the gradual coevolution of defense-related chemistry—a so-called arms race between prey and predators. This arms race is a dynamic process that contributes to adaptive evolution of both plants and pests and has led to the

isolated from environmental DNA. This suggests that such genes might be widespread among microorganisms and that bacteria might be one source of *N*-acyl glutamines involved in plant–insect interaction. Indeed, bacteria in the oral secretion from *S. exigua* larvae are capable of producing *N*-acyl amino acids [9]. The

same study demonstrated that other bacteria isolated from the insect gut could synthesize *N*-linolenoyl glutamines when supplied with linolenic acid and glutamine [9], but the insect host also seems to possess a gut enzyme that catalyzes the conjugation of food-derived fatty acids with glutamine.

wide variety of plant chemical defenses, including countless toxins and other bioactive compounds. This cornucopia of chemicals offers huge opportunities for biomedical research, drug development, and human health: Many pharmaceutically relevant compounds derived from plants—including digitoxin, morphine, artemisinin, or taxol—evolved in the context of plant defense. The ongoing arms race between plants and their attackers will continue to drive the generation of new compounds, while associated microbes contribute to the chemical talk in plant–insect interactions.

Moreover, better knowledge of the defensive mechanisms and the relevant compounds will provide important tools for agriculture. Traditional breeding or bioengineering could create plant varieties that produce additional repellents, toxins, and other protective compounds, thereby enabling a particular crop to better withstand herbivore and pathogen attacks. In particular, indirect defense that involves the recruitments of organisms at the third trophic level could be an interesting approach to reduce the use of insecticides in agriculture. One tool for integrated pest management programs is the so-called push-pull strategy [10]. This approach uses a combination of chemical signals to manipulate both the distribution and abundance of insect pests and their natural enemies. The pest insects are repelled or deterred away from the main crop (push) by using stimuli that either mask the plant or are repellent or deterrent. Simultaneously, pests are attracted to and concentrated in other areas (pull) by attractive signals from trap crops. Pilot projects using the push-pull strategy have been carried out by scientists of the International Centre of Insect

Physiology and Ecology (ICIPE) in Kenya and Rothamsted Research in the UK, as well as other research organizations in Eastern Africa, where at least four species of stem borers infest maize and sorghum crops and result in yield losses of 10–50% [10]. These projects have involved the combined use of intercrops and trap crops, the latter using plants that were appropriate to the farmers, and contributed to increased crop yields and livestock production [10].

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Strikingly, this strategy is not new. More than 500 years ago, the Inka in the Andes of South America protected their potato plants from pests by surrounding the fields with mashua plants (*Tropaeolum tuberosum*), which have strong antimicrobial, insecticide, and nematicide capacities. Nevertheless, to efficiently use and improve such strategies, we need to better know the chemical signals at the different trophic levels. In other words, we need to decipher and learn the chemical language used by plants, herbivores, and predators or parasitoids. Let us learn to speak chemistry; let us learn Entish.

Conflict of interest

The authors declare that they have no conflict of interest.

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