Air pollution impedes plant-to-plant communication, but what is the signal?

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Since the first reports that undamaged plants gain defensive benefits following exposure to damaged neighbors, the idea that plants may signal to each other has attracted much interest. There has also been substantial debate concerning the ecological significance of the process and the evolutionary drivers. Part of this debate has centered on the distance over which signaling between plants occurs in nature. In a recent study we showed that an ozone concentration of 80 ppb, commonly encountered in nature, significantly reduces the distance over which plant-plant signaling occurs in lima bean. We went on to show that degradation of herbivore-induced plant volatiles by ozone is the likely mechanism for this. The key question remaining from our work was that if ozone is degrading the signal in transit between plants, which chemicals are responsible for transmitting the signal in purer air? Here we present the results of a small scale experiment testing the role of the two most significant herbivore-induced terpenes and discuss our results in terms of other reported functions for these chemicals in plant-plant signaling.

Plant-to-plant signaling mediated by volatile chemical compounds has been reported in numerous studies conducted under both laboratory^{1,2} and field^{3,4} conditions. It is one of the most sensitive volatile-mediated processes in nature and consequently the ecological significance of the phenomenon has been frequently questioned.⁵ In addition, the evolutionary advantage of this process to the signal

emitting plant is rather unclear, although several non-exclusive suggestions have recently been made.⁵ Despite the remaining uncertainties, there is now little doubt that plant-plant signaling exists, and that it can be observed under field conditions.

A range of volatile chemicals have been implicated as providers of inter-plant signals, including phytohormones such as methyl jasmonate, methyl salicylate and ethylene, various terpenes and green leaf volatiles (GLVs).⁶ However, most of the underlying mechanisms, particularly concerning the perception of signal molecules, remain to be elucidated.⁵ One of several outstanding questions related to airborne plant-plant signaling concerns the distance over which the signals are effective. The volatile chemicals that transmit signals between plants must remain intact and at sufficient concentration to be detected and elicit a response in receiver plants. The distances over which signal chemicals can function are limited by abiotic factors such as wind speed, air humidity and temperature.⁵ With respect to plant-plant interactions, much has been made about the effects of signal dilution in air currents,^{5,7} but the degradation of the signaling compounds by reactive atmospheric pollutants can also represent a major obstacle to efficient plant-plant signaling.⁶ The same can be said about other volatile mediated interactions including the attraction of predators, parasitoids and pollinators, and indeed some effects of ozone on foraging by parasitoids has been observed.⁸ In a recent study, we showed that 80 ppb ozone significantly reduces the distance over which plant-plant communication is

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Figure 1. Volume of nectar secreted from nectaries following treatment with DMNT + ocimene (DO) or control (0.1% Tween) solutions. Nectar secreted is expressed in microlitres per gram of dried leaf mass for the first trifoliolate leaves (TL), primary leaves (PL) and the whole plant. Differences between treatments were tested with independent samples t-tests; the p value for each comparison is given above the bars.

effective.⁹ We concluded that the mechanism for this was the rapid degradation of volatile chemicals during their transit between plants.

Herbivore-induced plant volatiles (HIPVs) may influence the defence characteristics of receiver plants through active and passive processes.^{10,11} The active process is where the receiver plant actively changes in response to a signal, for example gene activation^{2,12} or increased production of extra-floral nectar (EFN).⁴ This is true plant-plant signaling involving perception of a signal. The passive process is where volatile chemicals adsorb to the surfaces of plants and affect plant defence either as a result of their subsequent re-volatilization or their enduring presence on the surfaces of undamaged receivers. This process does not require perception. Both of these processes have been demonstrated to have ecological significance in field studies.^{3,4,10} The reactions of volatile chemicals with pollutants can influence both processes in different ways. It is most likely that the active process will be rendered ineffective, while the passive process may be more complexly altered with volatile

degradation products potentially adsorbing to surfaces with subsequent effects on other organisms. We previously examined the effects of ozone on active plant-plant communication and used secretion of EFN as a measurable defence trait.⁹ As ozone successfully reduced the distance over which communication occurred we can predict that reaction products are unlikely to function as suitable signals in active plant-plant communication, while it has previously been indicated that they are also ineffective as volatile cues to foraging parasitoids.⁸

GLV (*Z*)-3-hexenyl acetate has previously been implicated as a key inter-plant signal in lima bean (*Phaseolus lunatus*), with an inductive effect on secretion of EFN.⁴ In our study⁹ (*Z*)-3-hexenyl acetate was a relatively minor component of the mite-induced volatile bouquet. We mixed HIPVs with clean air or ozone enriched air in 22.3 L glass reaction chambers and did not recover (*Z*)-3-hexenyl acetate in either treatment, suggesting that the quantities emitted were so low that they were quickly diluted to below detection levels in ambient air. We found that two of the dominant

HIPVs, (*E*)- β -ocimene and (*E*)-4,8-dimethyl-1,3,7-nonatriene [(*E*)-DMNT], both previously shown to be induced by spider-mite feeding in lima bean,^{2,4} were present in ambient air, but broken down rapidly by ozone. Therefore, we conducted a small scale experiment to re-evaluate their potential roles as signaling molecules in plant-plant communication.

A solution containing 25 μ l of (*E/Z*)-DMNT (1:1 ratio of the isomers) and (*E*)- β -ocimene (volume ratio: 6:4) dissolved in 24.975 ml of 0.1% Tween20 in 5% ethanol was made. Plants, 18 per treatment, were randomly assigned to control or DMNT + (*E*)- β -ocimene (DO) treatments using the random number generator in Microsoft Excel. The DO solution was sprayed once onto each primary leaf of 2-week-old lima bean seedlings with a handheld sprayer; the spray projection also covered the terminal shoot. Control plants were sprayed with 0.1% Tween20 solution. Applications corresponded to 1.2 ml of solution per spray. This technique of applying formulations to plants has previously been used to apply methyl jasmonate,¹³ with successful elicitation of plant responses. Plants were left to dry for 15 min before transferring them to controlled environment chambers (Weiss Bio 1300; Weiss Umwelttechnik GmbH, Reiskirchen-Lindenstruth, Germany). After three days, the volume of nectar secreted by plants was quantified in graduated 5 μ l Hirschmann microcapillary pipettes (Sigma-Aldrich Chemie GmbH, Munich, Germany), with the nectar secreted by nectaries on the primary leaves and nectar secreted by nectaries on the trifoliolate leaves collected separately. For a subset of plants the sugar content of the nectar was analyzed by High Performance-Liquid Chromatography (HPLC Agilent 1100 Series, Waldbronn, Germany). In brief, a 5 μ l aliquot of EFN extract was injected into an Agilent Zorbax Carbohydrate Analysis column (150 mm x 4.6 mm i.d., 5 μ m film thickness), eluted isocratically with 75% acetonitrile/25% Milli-Q water at a flow rate of 1.5 ml min⁻¹ and monitored by a refractive index detector (hp1037A, Hewlett-Packard, Wilmington, DE). The sugars (fructose, glucose and sucrose) were identified and quantified by comparing their retention times and peak areas with pure standards.

Figure 2. Sugar content of extra-floral nectar secreted by whole plants treated with DO or control solutions. The amount of each sugar present is presented in micrograms per gram of leaf dry mass. Differences between treatments were tested with independent samples t-tests; the p value for each comparison is given above the bars.

We found no significant effect of DO application on volume of EFN secreted (Fig. 1) or sugar content (Fig. 2). This observation is consistent with previous work in reference 4, in which lima bean plants were exposed to DMNT or ocimene released from lanolin paste with no significant effect on EFN soluble sugars. However, the sample sizes in this earlier study were rather low, and a greater quantity of sugar was secreted after DMNT treatment, even though it was not significantly greater than controls. In a study with excised lima bean leaves,² exposure to each of these compounds resulted in the expression of several defense-related genes. Ocimene has also been found to induce an increase in tissue levels of methyl jasmonate and transcript levels of defence or stress related genes in *Arabidopsis*.¹² So it appears that both these compounds can play roles in plant-plant signaling, even if the respective roles are not related to EFN regulation. (*Z*)-3-hexenyl acetate remains

the sole compound to be linked directly to EFN secretion through plant-plant signaling, and has also been shown to play a role in within-plant signaling via volatile compounds.¹⁴ If (*Z*)-3-hexenyl acetate is the main active signaling molecule modulating EFN responses, we can conclude that it must be active at very low concentrations.

In summary, the distance over which plant-plant signaling occurs is significantly reduced by ozone pollution. Many HIPVs, including (*E*)- β -ocimene, (*E*)-DMNT and (*Z*)-3-hexenyl acetate, are rapidly degraded by ozone. Neither (*E*)-DMNT nor (*E*)- β -ocimene significantly alter EFN secretion in receiver plants. It is possible that (*Z*)-3-hexenyl acetate, as indicated in previous studies in references 4 and 14, is the active signaling compound. However, as no mechanism has been elucidated to explain this process, other reactive HIPV may well be involved. Further work is clearly required to shed more light on this issue.

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