RECURRING EFFECTS OF AGRICULTURAL CHEMICALS ON FLORAL ELECTRIC FIELDS

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Abstract: Although adverse effects of pesticides on ecological interactions are frequently studied and well recognized, their potential effects on the interaction between biological organisms, natural electric potentials and charge distributions are currently overlooked. Since most pesticides and fertilizers carry an electric charge, it is conceivable that agricultural chemicals affect the electrical potential of plants, and putatively the interactions of flowers with their environment, including their pollinators. Following pesticide application, subsequent rain events could potentially wash out, or alter these interactions. Therefore, we set out to assess the effects of both pesticides and fertilizers on flower electric fields and evaluate persistence of agricultural chemical-induced changes in floral electric fields after subsequent rain events in a laboratory setup. Results indicate that agricultural chemicals have the potential to substantially affect a floral electric field for long time periods, and that subsequent rain events can trigger a legacy response of floral electric fields previously treated with agricultural chemicals. This implies that spray applications of agrochemicals can have persistent direct and indirect adverse effects on plant electric ecology, electrophysiology and interactions with associated fauna.

Introduction

Flowers often exhibit a negative electric potential (Corbet et al., 1982), arising from the potential of the flower in relation to the atmospheric electric field. These electric fields (E-fields) can be relevant for ecological interactions at wider spatial and temporal scales. Bumblebees, for instance, can detect and learn to use floral electric fields, and their structural variation, to assess floral reward and discriminate among flowers (Clarke et al., 2013). The ubiquity of E-fields in nature and their relevance for plant-insect interactions thus suggest that E-fields are an essential, yet understudied ecosystem component that could be prone to anthropogenic stressors. Such stress factors could conceivably be particularly prevalent in agricultural and floricultural dominated ecosystems.

Agricultural practices inevitably involve the intensive use of pesticides and fertilizers to increase crop yields and efficiency of production processes (Hossard et al., 2014), despite its various direct and indirect adverse ecological effects on ecosystems and the organisms living therein (e.g. Kessler et al., 2015; Tison et al., 2016; Hunting et al., 2016, 2017; Schrama et al, 2017). The adhesive nature of pesticides often relies on the fact they carry an electric charge, generating electrostatic particles that readily associate with the plant surface (e.g. Tomizawa et al., 2000; 2003; Islam et al., 2017) where it potentially affects its dielectric and electrophysiological properties. These electrical interactions are likely further complicated by subsequent absorption of these chemical by the plant, or meteorological phenomena such as rain events or solar radiation that may either dilute or sensitize adsorbed and absorbed chemicals. It thus seems conceivable that, ultimately, there is a complex interplay between agricultural chemicals and plant electrophysiological properties that may extend to wider physico-chemical and ecological interactions, yet a comprehensive understanding thereof is currently lacking.

Since most pesticides and fertilizers carry a charge, it is conceivable that agricultural chemicals can affect the electrical properties of the flower. This study therefore aims to 1) assess effects of both pesticides and fertilizers on flower E-fields and 2) evaluate the persistence of agricultural chemical-induced changes in floral electric fields in the context of potentially relevant meteorological phenomena, including rain events and solar radiation. To this end, floral E-fields were monitored during different spray-scenarios in a simplified laboratory setting.

Methods

Experimental set up – Flowers (*Eustoma russellianum*) where cut halfway down their stem, rooted in water and placed in a Faraday-cage. Electric fields of flowering plants were subsequently assessed using a sharpened tungsten needle electrode positioned approx. 10 cm below the flower corolla, as described in detail by Clarke et al., 2013. The electrode was connected to an extracellular voltage amplifier (WPI DAM-50), grounded to an independent earth. The flower under test was not grounded to the amplifier. Data acquisition was performed with NI acquisition card (model xyz) feeding into a Toshiba laptop computer. Voltage shown here refer to amplified responses, and not in situ voltage recordings.

Experimental design – The experimental spray application was designed to test for the repeated effects of demineralized water (dH_20) on flower electric fields, a subsequent spray application effect of either a charged pesticide or mixture of fertilizers, and a subsequent rain event, mimicked by spraying (dH_20). Hence, flowering plants were subject to a sequential spray application, including 1) dH_20; 2) dH_20; 3) dH_20; 4) Pesticides/Fertilizers; 5) dH_20, in which both pesticides and fertilizers were considered treatments. Both treatments were replicated four times. Prior to assessing effects of agricultural chemicals on plants electric fields, electrical responses of an aluminum plate to the application of demineralized water (dH_20) and nutrients were assessed to control for the non-biological, electro-physical responses of the experimental set up (Fig. 1).

Data analysis – Each spraying event resulted in a distortion of the electric field (Fig. 2; small arrows). Depending on the treatment, some time elapsed before its natural electric field was restored (Fig 2; striped arrow). The time-elapsed for electric field recovery was calculated after each spraying event, considering **1)** the replicated dH_20 before the pesticide/fertilizer treatment; **2)** the replicated pesticide/fertilizer treatment; and **3)** the mimicked rain event (replicated dH_20) after the pesticide/fertilizer treatment. Data was tested for normality of distributions using a Shapiro-Wilkinson test, and differences between time-elapsed to restored fields were subsequently detected with a 1-way ANOVA and a Tukey's pairwise comparison.

Results

A representative plot of electrical field changes of an aluminum plate in response to the application of demineralized water (dH20) and nutrients is depicted in Fig 1, showing short recovery times for E-fields after spray applications, irrespective of treatment. Response traces were not filtered prior to acquisition and are shown as raw files here. In effect, electrical noise was observed during all experimental runs and was confirmed to be local AC 50Hz of the main supply.

Plant response

A representative run of experimental spray application on a flowering plant is presented in Fig. 2. Repeated effects of demineralized water (dH20, blue arrows) on flower electric fields, a subsequent spray application effect of either a charged pesticide or mixture of fertilizers (red arrow), and a subsequent rain event, mimicked by spraying (dH20). Time elapsed for recovery of E-field (dashed arrow) was used to assess treatment effects (Fig 3a,b).

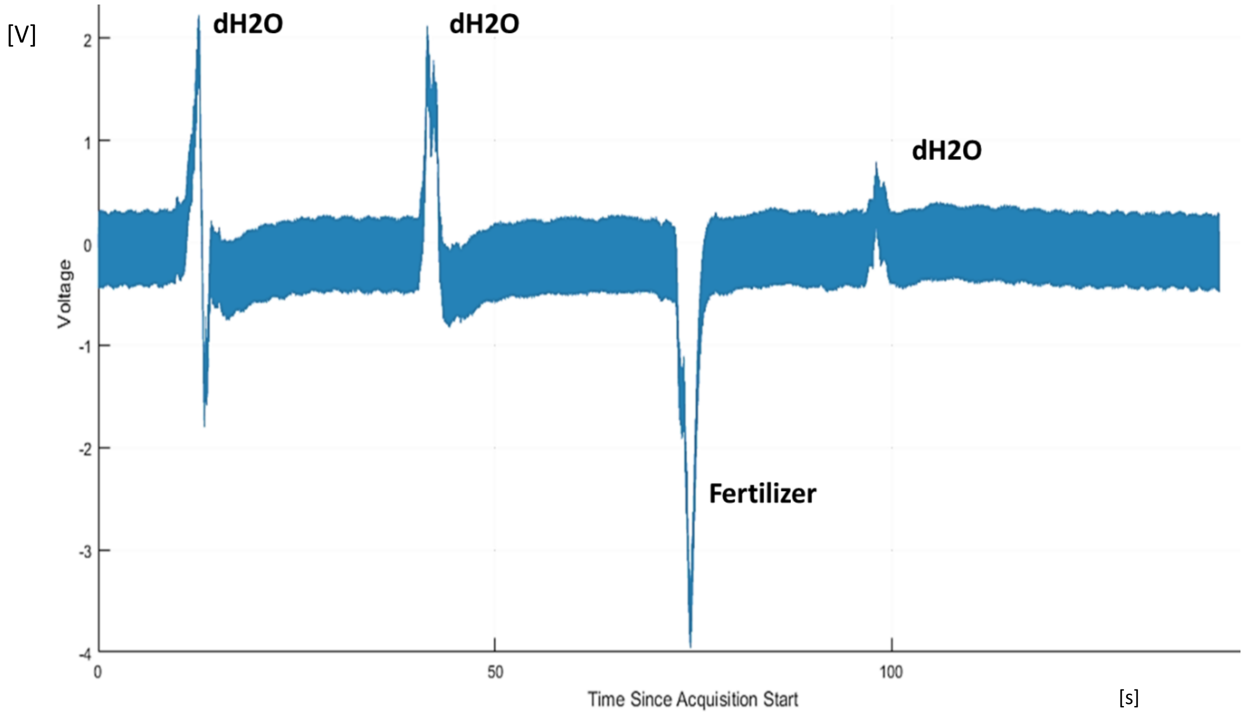
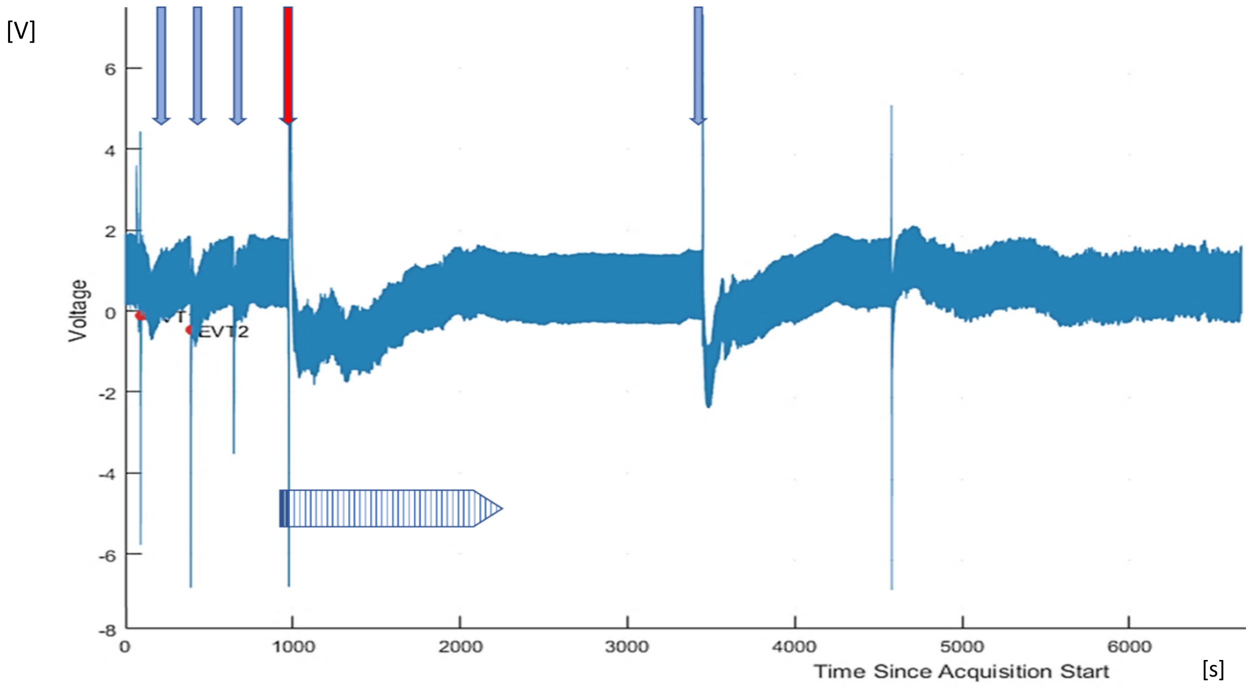


Figure 1: Electrical field changes of an aluminum plate in response to the application of demineralized water (dH20) and nutrients were assessed to control for the non-biological, electro-physical responses of the experimental set up.

Figure 2: Representative run of experimental spray application to test for the repeated effects of demineralized water (dH20, blue arrows) on flower electric fields, a subsequent spray application effect of either a charged pesticide or mixture of fertilizers (red arrow), and a subsequent rain event, mimicked by spraying (dH20). Flowering plants were subject to a sequential spray application, including 1) dH20; 2) dH20; 3) dH20; 4) Pesticides/Fertilizers; 5) dH20. Time elapsed for recovery of E-field (dashed arrow) was used to assess treatment effects.



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Application of pesticides resulted in a significantly prolonged time for E-field recovery compared to the initial control spray application of dH2O (Fig. 3a – 1-w. ANOVA, df 2; F46,65; p<0,0001). Likewise, application of fertilizers resulted in a significantly prolonged time for E-field recovery compared to the initial control spray application of dH2O (Fig. 3b – 1-w. ANOVA, df 2; F29,77; p<0,0001). Simulating a rain event with dH2O post-treatment of both pesticides and fertilizers resulted in a prolonged period for E-field recovery (Fig. 3 a and b).

The use of solar lamps resulted in excessive electrical noise. This noise is unwanted as it contaminates electrical recordings, but also constitutes an undesirable putative stimulus to the plant and chemical application. This issue could not be resolved at the time of experimentation but is solvable in the future. It was observed that short (1 – 5 minutes) exposure did not result in massive changes in treatment effects on plant E-fields, although in some cases E-field recovery periods seemed markedly prolonged. Time-constraints prevented full experimentation, including noise-free controls, and hence firm conclusiveness.

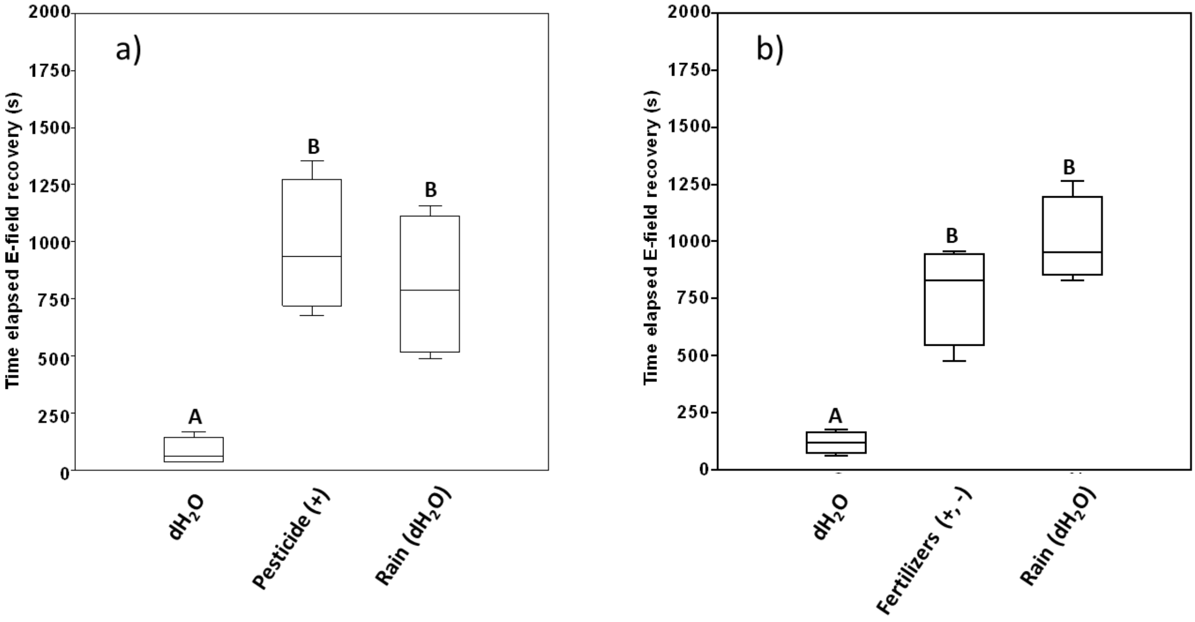

Figure 3: Time required to recover the E-field of the flowering plant after spray events of either demineralized water (dH2O), pesticide (a) and fertilizer (b) treatments, and mimicked rain events (dH2O). Corresponding letters (A+B) indicate statistical similarity (one-way ANOVA, Tukey's post hoc test, $p < 0.05$).

Discussion & Conclusion

We exposed flowering plants to two types of agrochemicals. Actual mechanisms for the observed flower electric field changes were not assessed and hence remain uncertain in the current set up. Charged particles likely have the potential to directly affect the surface electrochemistry and therefore electric potential of the flower. Plants are known to elicit electrophysiological responses to a variety of stressors, including chemical, mechanical, photonic (e.g. Maffei & Bossi, 2006), while E-field changes can result in fitness responses of the plant itself (e.g. Murr, 1963). Irrespective of the underlying mechanisms, our preliminary results clearly indicate that agricultural chemicals have the potential to affect a floral electric field for substantial periods of time.

While spray applications with demineralized water did not result in substantial periods of E-field changes, demineralized water resulted in significant periods of E-field alterations when applied after a spray application of both pesticides and fertilizers. This is likely due to solubilization of adsorbed charged particles present on the flower's surface, and subsequent direct alterations of the flower's E-field and indirect electrophysiological responses of the plant. While the use of solar lamps did not provide conclusive results in this experimental period, it is well known that solar radiation, and in particular UV-radiation, strongly contributes to the degradation of pesticides over hours- to weeks-long time periods (Černigoj et al., 2010; Rózsa et al., 2017). These degradation products often carry a charge as well, and hence likely contribute to affect, if not complicate, the electrical properties of flowers treated with agrochemicals. Current pesticide spraying guidelines dictate specific time-frames (evenings) where pesticides may be applied to prevent immediate adverse effects on non-targeted organisms. Data presented here, however, suggests that direct adverse effects of pesticides may also be re-established throughout subsequent rain events. The possible presence of such effect generates novel questions as to how and why such longer-term effects may be present, and what their effects are on the electric ecology of plants and their pollinators.

In addition to direct adverse effects, substantial prolongation of flower electric field alteration and associated rain-induced E-field alterations due to spray application of both pesticides and fertilizers could potentially results in indirect ecological effects at wider spatial and temporal scales. Pollinating honeybees, for instance, usually possess a positive electric potential, while flowers often exhibit a negative potential (Corbet et al., 1982). Electric fields arising as a result of this potential difference between flowers and insects promote pollen transfer and adhesion over short distances. The electrical interactions between the bee and the flower arise from the charge carried by the bee and the potential of the flower in relation to the atmospheric electric field. Bumblebees can detect and learn to use floral electric fields, and their structural variation, to assess floral reward and discriminate among flowers (Clarke et al., 2013). The ubiquity of electric fields in nature and their integration into the bees' sensory ecology suggest that E-fields play a crucial role in plant-insect interactions (Clarke et al., 2013). The electric interactions between agrochemicals and flowers observed here likely also affects the electrostatic interaction between bees and flowers, and thus could ultimately affect floral choices of bees, thereby posing a potential mechanism underlying observed negative impacts of pesticides on bee behavior. However, it remains uncertain how pesticides can affect flower choices of bees and hence a fundamental understanding of pesticide effects on bee-flower interactions is urgently required.

Although adverse effects of pesticides in ecological interactions are frequently studied and well recognized, potential biology-electric field cross-over effects are currently overlooked. This project aimed to assess effects of both pesticides and fertilizers on flower electric fields and to evaluate persistence of agricultural chemical-induced changes in floral electric fields in the context of potentially relevant meteorologically phenomena, including rain events and solar radiation. Results indicate that agricultural chemicals have the potential to affect a floral electric field for substantial periods, and that subsequent rain events can trigger a legacy response of floral electric fields previously treated with agricultural chemicals. Although these results were preliminary and obtained in a simplified setting, this study yielded preliminary data on the potential of agricultural chemicals affecting natural electro-ecological interactions, and may have wider implications for electrical interactions in ecosystems, for instance a bumblebee's ability to sense electric fields and inherent floral choice, and foraging and pollinating behavior.

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