Mechanosensitivity below Ground: Touch-Sensitive Smell-Producing Roots in the Shy Plant *Mimosa pudica*[1][OPEN]

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The roots of the shy plant *Mimosa pudica* emit a cocktail of small organic and inorganic sulfur compounds and reactive intermediates into the environment, including SO_2, methanesulfinic acid, pyruvic acid, lactic acid, ethanesulfinic acid, propanesulfenic acid, 2-aminothiophenol, *S*-propyl propane 1-thiosulfinate, phenothiazine, and thioformaldehyde, an elusive and highly unstable compound that, to our knowledge, has never before been reported to be emitted by a plant. When soil around the roots is dislodged or when seedling roots are touched, an odor is detected. The perceived odor corresponds to the emission of higher amounts of propanesulfenic acid, 2-aminothiophenol, *S*-propyl propane 1-thiosulfinate, and phenothiazine. The mechanosensitivity response is selective. Whereas touching the roots with soil or human skin resulted in odor detection, agitating the roots with other materials such as glass did not induce a similar response. Light and electron microscopy studies of the roots revealed the presence of microscopic sac-like root protuberances. Elemental analysis of these projections by energy-dispersive x-ray spectroscopy revealed them to contain higher levels of K^+ and Cl^- compared with the surrounding tissue. Exposing the protuberances to stimuli that caused odor emission resulted in reductions in the levels of K^+ and Cl^- in the touched area. The mechanistic implications of the variety of sulfur compounds observed vis-à-vis the pathways for their formation are discussed.

Plant roots are known to exude a diversity of both small and macromolecular chemicals that mediate antimicrobial, antiquorum sensing, allelopathic, and other effects (De-la-Peña et al., 2012). However, the machinery associated with the synthesis and extrusion of these compounds is not well understood. One of the most intriguing but least studied of these is the emission of volatile and reactive organosulfur compounds such as the foul and toxic gas carbonyl sulfide (COS) and volatile carbon disulfide (CS_2). Both are reportedly released by numerous plants and are proposed to make a significant contribution to the environmental sulfur burden (Haines et al., 1989). As a case in point, the Central American rainforest plant *Stryphnodendron exelsum* (Mimosaceae) is a sufficiently strong sulfur emitter that its location in the forest can be determined by odor (Haines et al., 1989). Furthermore, 40 taxa from nine genera within the subfamily Mimosoideae revealed that 29 taxa from six genera produced CS_2 and 19 of the 40 taxa produced COS (Piluk et al., 2001). It has been proposed that the COS and CS_2 are derived from a putative Cys lyase-mediated cleavage of djenkolic acid, an amino acid previously isolated from the plant (Piluk et al., 1998), but this has not been confirmed.

We used *Mimosa pudica* (Leguminosae), a perennial shrub endemic to Brazil but now pantropical in its distribution (Howard, 1988), as a model to begin investigations of how this and related plants emit these highly reactive and corrosive compounds without themselves incurring tissue damage. Its various colloquial names, such as sensitive plant, touch-me-not, shy plant, and humble plant, among many others (Holm, 1977), derive from its seismonastic movements: in response to touch, water, shaking, wind, or warming, its leaves quickly close, slowly opening after an average of about 10 min (Song et al., 2014). It also displays nyctinasty, with its leaves closing or "sleeping" with the onset of darkness. These curious characteristics coupled with its small size have made the plant a convenient and popular attraction in schools, greenhouses, and other learning environments, where it is used to illustrate seismonasty.

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R.A.M. conceived the work, designed the experiments, conducted experiments, interpreted the data, and wrote the article; A.D.L. and R.B.C. conducted mass spectrometric measurements, and R.B.C. interpreted some of the resulting data; M.J.M. conducted GC-MS and various control experiments; D.E. conducted microscopy experiments; K.L.F. performed growth chamber MS headspace analysis experiments and germinated seedlings; A.J.D. conducted GC-MS experiments; M.C.L. germinated plant seedlings.

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Our studies show that by using direct analysis in real time high-resolution mass spectrometry (DART-HRMS; Cody et al., 2005), it is possible to detect the compounds emitted by plant roots in situ. Using this method, it was revealed that both *M. pudica* plants germinated aseptically on agar and those germinated in soil emitted a variety of small molecules into the atmosphere at levels that were not detectable by human subjects. However, an odor detectable by humans could be sensed when the plant root was disturbed, with odor emission being dependent on the nature of the stimulus. Analysis of the chemical contributors to the odor revealed that, although the array of compounds observed to be produced by the roots was the same both before and after stimulation, emission of a subset of organosulfur compounds was increased when the roots were stimulated. Light and scanning electron microscope imaging studies revealed the presence of sac-like protuberances dotted along *M. pudica* seedling root shafts that collapsed when the roots were exposed to stimuli that elicited odor emission. The detection by energy-dispersive x-ray spectroscopy (EDS) of relatively high levels of K^+ and Cl^- prior to root stimulation, on the one hand, and reductions in the levels of these species, on the other hand, implicate the involvement of these ions in the observed mechanostimulatory behavior.

RESULTS

M. pudica Seedlings Emit Organosulfur Compounds into the Environment

In previous studies where odor emission from *M. pudica* roots was reported (Hartel and Haines, 1992; Hartel and Reeder, 1993; Piluk et al., 1998), roots from gnotobiotically grown plants were detached from the aerial parts, washed with water, and subsequently crushed in an air-tight plastic syringe. After a 7-min delay, the headspace of the crushed roots was analyzed by gas chromatography-mass spectrometry (GC-MS). The only compound detected by this method was CS_2; therefore, it was concluded that the compound responsible for the odor detected when *M. pudica* is uprooted was CS_2.

From these studies, it remained unclear whether the CS_2 observed was emitted by the roots in situ or appeared as a consequence of the root tissue breach. Therefore, we first conducted headspace analysis of intact *M. pudica* seedlings to determine whether CS_2 was present in the absence of tissue rupture and to determine the optimal conditions for its detection by DART-HRMS. For these experiments, *M. pudica* seeds were germinated aseptically on agar so that they could be handled without tearing the roots. Seeds began germinating within 2 to 3 d, and seedlings grew to approximately 23 mm in length by the end of the first week. Over that time frame, each plant produced a single tap root that did not have hairs visible to the naked eye (Supplemental Fig. S1). Using sterile stainless steel tweezers, seedlings were transferred to sterile vials equipped with septum caps (one seedling per vial; Supplemental Fig. S2). In each case, the tweezers were used to grip the seedling at the hypocotyl. The transfer was accomplished in approximately 10 s. The seedling headspace was then immediately sampled for 5 min using a polydimethylsiloxane (PDMS) solid-phase microextraction (SPME) fiber (Supplemental Fig. S2), and the fiber was subsequently analyzed by DART-HRMS in both positive and negative ion modes. Representative results are shown in Figure 1. The positive ion mode mass spectrum (Fig. 1A) included peaks at nominal mass-to-charge ratio (*m/z*) 93, 110, 167, and 184, whose exact masses corresponded to formulas C_3H_9OS, C_6H_8NO, $C_6H_{15}OS_2$, and $C_6H_{18}NOS_2$, respectively. The formulas that contained sulfur were consistent with those of a number of organosulfur compounds common to *Allium* spp. such as onion (*Allium cepa*), most notably propane sulfenic acid (*m/z* 93) and *S*-propyl propane 1-thiosulfinate in both protonated and ammoniated forms (*m/z* 167 and 184, respectively). The thiosulfinate serves as the major odor and flavor molecule produced in freshly cut onions, and the sulfenic acid is the reactive intermediate precursor of the thiosulfinate. The identity of the thiosulfinate was confirmed by comparing the DART-HRMS mass spectral fragmentation patterns of authentic standards obtained under in-source CID conditions (cone voltage of 90 V) with fragments observed by DART-HRMS analysis of the *M. pudica* root samples under similar in-source CID conditions. As sulfenic acids are fleeting reactive intermediates that cannot be isolated, it was not possible to confirm the structural identity of the peak at *m/z* 93. Thus, the propane sulfenic acid structural assignment is putative, albeit informed by the observations outlined in published studies showing that this sulfenic acid is the direct precursor of the *S*-propyl propane 1-thiosulfinate observed in this work and also seen in onion (Block, 1992). Furthermore, Block et al. (2010, 2011) have observed this intermediate in onion using DART-HRMS.

Figure 1B shows the DART-HRMS results of headspace analysis of the seedling in negative ion mode. Notable peaks included those at nominal *m/z* 60, 61, 91, 124, 165, and 198, whose exact masses corresponded to formulas CO_3^-, HCO_3^-, C_3H_7SO, C_6H_6NS, $C_6H_{13}OS_2$, and $C_{12}H_8NS$, respectively. Formula C_3H_7SO is consistent with the presence of the deprotonated counterpart of the sulfenic acid intermediate putatively identified in the positive ion mode spectrum shown in Figure 1A. However, as stated previously, its identity cannot be confirmed because it is a reactive intermediate, as reported on extensively by Block (1992). While $C_6H_{13}OS_2$ corresponded to the deprotonated form of the thiosulfinate observed in the positive ion mode spectrum, C_6H_6NS was consistent with that of an aminothiophenol (*ortho*, *meta*, or *para*), and the $C_{12}H_8NS$ corresponded to phenothiazine. In order to confirm

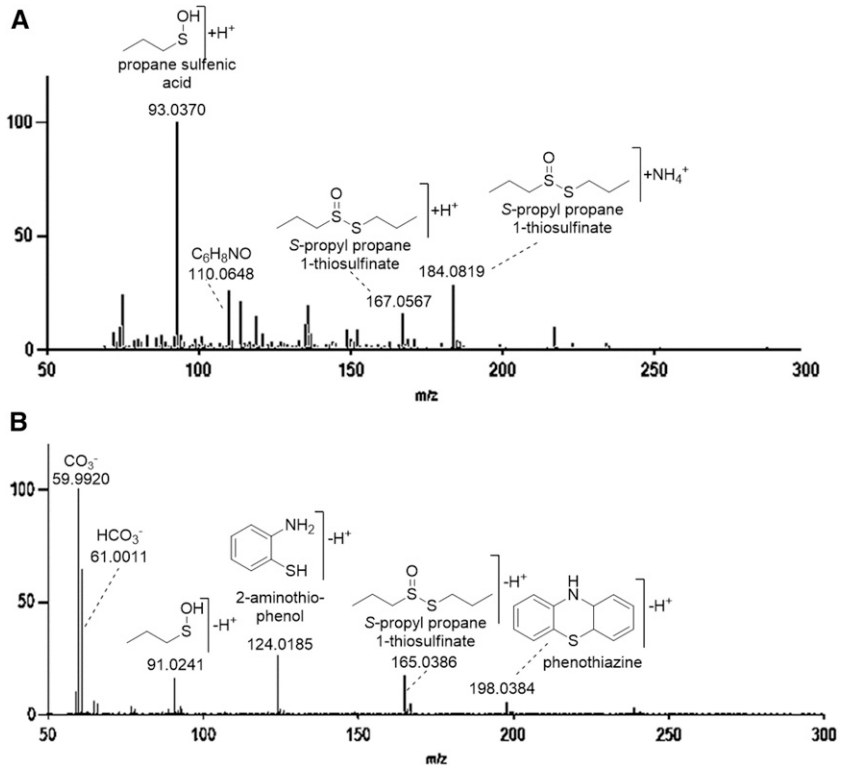

Figure 1. Typically observed DART-HRMS positive (A) and negative ion mode (B) spectra of the headspace of 7-d-old *M. pudica* seedlings in the absence of an odor-producing stimulus. In each case, a SPME fiber was exposed to the headspace for 5 min, and the fiber was then analyzed by DART-HRMS. The structures shown are consistent with the observed high-resolution elemental compositions and isotope data obtained as well as the results of comparisons of the fragmentation patterns observed for standards under in-source collision-induced dissociation (CID) conditions with those of the headspace samples also obtained under in-source CID conditions. Detected compounds were observed in their protonated or ammoniated form. The mass measurements and relative peak abundances associated with the data shown here are presented in Supplemental Table S1.

these tentative structural assignments, authentic standards of *ortho*-, *meta*-, and *para*-aminothiophenol, as well as an authentic standard of phenothiazine, were subjected to in-source CID by DART-HRMS in negative ion mode. The fragmentation patterns were then compared with the *M. pudica* seedling spectrum acquired under identical conditions. The fragmentation patterns observed showed that C_6H_6NS and $C_{12}H_8NS$ corresponded to *ortho*-aminothiophenol (also known as 2-aminothiophenol) and phenothiazine, respectively.

It Was the Roots and Not the Aerial Parts of *M. pudica* That Emitted Organosulfur Odor Volatiles

In order to determine whether *M. pudica* aerial parts contributed to the organosulfur volatiles profile, a method was devised to permit analysis of the roots and aerial parts separately in a manner that prevented the disruption of plant tissue. Under sterile conditions, a bed of agar was suspended within a glass cylinder (Supplemental Fig. S3A). The bottom of the cylinder was sealed with a septum and sterile water was introduced (via syringe), such that an air pocket remained between the agar and the water surface (Supplemental Fig. S3C). Deposition of a 3-d-old aseptically germinated *M. pudica* seedling on the top surface of the agar within the vertically mounted cylinder resulted in downward growth of the root through the agar plug toward the water (Supplemental Fig. S3C). Within 48 h, the root eventually emerged from the bottom of the agar, so that it was freely suspended in the open air space between the bottom of the agar disc and the water level, without touching the water, while the aerial part grew above the agar bed. In this way, the agar served to separate the compounds emitted by the aerial and root parts and allowed them to be analyzed independently. The root headspace was sampled with a PDMS SPME fiber by withdrawing the water from the bottom of the glass cylinder and inserting the SPME fiber as described earlier. The aerial headspace was sampled by sealing the top of the glass receptacle and inserting the SPME fiber as described. Representative negative ion mode DART-HRMS spectra of the headspace of the separated *M. pudica* aerial and root parts are shown in Figure 2, rendered in a head-to-tail plot format. The root headspace (top spectrum) showed a profile of compounds that was quite different from that detected in the aerial headspace (bottom spectrum). Notably, none of the compounds detected in the root headspace were observed in the aerial headspace, and vice versa. In addition, organosulfur compounds, including the propane sulfenic acid, 2-aminothiophenol, *S*-propyl propane 1-thiosulfinate, and phenothiazine detected in the DART-HRMS negative ion mode spectrum of the seedling (Fig. 1B), were observed. The results indicated that organosulfur compounds were emitted by the roots and not the aerial parts. Furthermore, since the analysis was conducted under sterile conditions and without breaching the plant tissue, neither the molecules detected in the aerial headspace nor those observed in the root headspace were contributions from intracellular components or microbes.

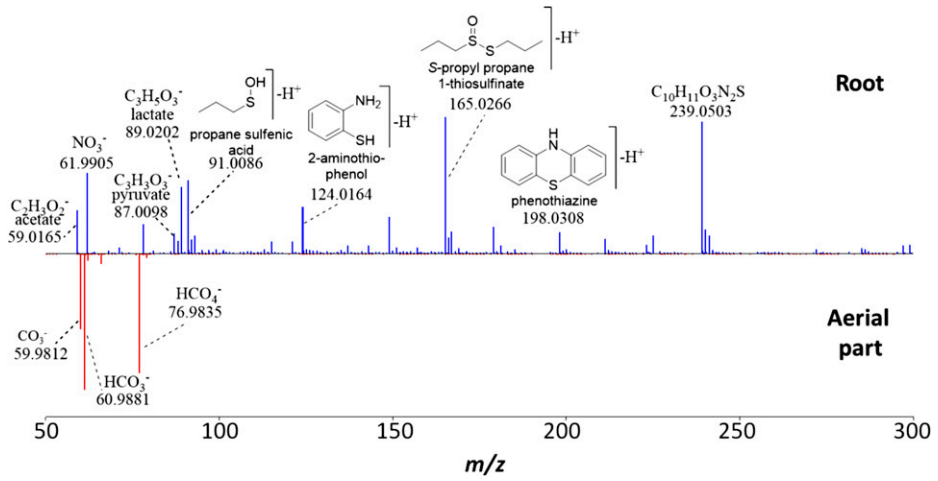

Figure 2. Head-to-tail plot of the typically observed negative ion mode DART-HRMS of the headspace gases produced by the root (top spectrum) and aerial part (bottom spectrum) of a 1-week-old *M. pudica* plant. The aerial and root parts were separated by an agar partition within a Pyrex tube. In each case, a SPME fiber was exposed to the headspace gases for 5 min, and the fiber was then analyzed by DART-HRMS. The structures shown are consistent with the observed high-resolution elemental compositions and isotope matching data as well as the results of in-source CID experiments. The mass measurements and relative peak abundances associated with the data shown here are presented in Supplemental Table S2.

M. pudica Roots Emit an Odor When Exposed to Certain Stimuli

In the course of these studies and in agreement with previous reports (Hartel and Reeder, 1993; Piluk et al., 1998), we detected a pungent, unpleasant sulfurous odor when 7-d-old gnotobiotically grown plants were dislodged from soil. However, more often than not, it was also observed that, when left undisturbed, neither seedlings germinated in soil nor plants germinated aseptically on agar exhibited an odor detectable to the human subjects performing the experiments. Furthermore, several human subjects reported that odor detection appeared to occur as a function of the exposure of seedling roots to some stimuli but not to others. For example, touching the roots with fingers often elicited a strong odor, while exposure of roots to glass (e.g. vials or stirring rods) or stainless steel (e.g. tweezers) did not. Because of these observations, a preliminary assessment of the presence or absence of an odor detectable to human subjects was conducted by a panel of five untrained subjects who were asked to indicate whether they detected a sulfurous odor when roots of 7-d-old seedlings gnotobiotically germinated on agar were touched. The sulfurous odor was defined as the smell the panelist experienced when an *M. pudica* seedling was dislodged from soil. The study was blind, in that the panelists were not apprised of whether the roots they were examining were touched or untouched. The study was performed by exposing the roots of 7-d-old seedlings to the following five stimuli: a finger; soil; glass; stainless steel; and wood. Panelists were allowed to smell the root within 15 s of root exposure to the stimulus and asked to indicate whether they experienced an odor different from agar. The experiments were performed in two ways. For all cases except exposure of the root to soil, the stimulus used was to tap the root once, as illustrated in Supplemental Video S1, where the root is tapped with a finger. The seedlings used were all germinated on the bed of agar. In the case of soil, the root was dragged across the soil surface, as illustrated in Supplemental Video S2, in order to simulate the effect of soil disruption that we and others observed resulted in odor release. Exposure of the seedlings to the various stimuli was conducted in replicates of five (i.e. each panelist was exposed to a total of five seedlings per stimulus experiment as well as to a control that was composed of a seedling germinated on agar that had not been touched with any stimulus). The results, shown in Supplemental Figure S4, revealed that, whereas root exposure to soil or fingers was observed to produce an odor detectable to the panelists most of the time (100% and 85% of the time, respectively), root stimulation with glass did not have that effect within experimental error. Odor detection by the panelists in response to the other stimuli occurred to varying extents, as indicated by the SD of the results (wood, 35 ± 19; and metal, 35 ± 25).

The DART-HRMS-Derived Headspace Profile of Compounds Emitted in Response to an Odor-Producing Stimulus Was Similar to That Observed in the Absence of an Odor-Producing Stimulus

In order to determine how the profile of compounds observed to be emitted by *M. pudica* seedlings in the absence of an odor-producing stimulus (Fig. 1) compared with that emitted by stimulated roots, the headspace volatiles of (1) 7-d-old sterile finger-stimulated

seedlings and (2) 3-month-old soil-bound plants in which the soil had been agitated by squeezing the pot three times were sampled by PDMS SPME and analyzed by DART-HRMS as described above. Examples of typically observed positive and negative ion mode mass spectra are shown in Figures 3 and 4, respectively. Positive ion mode spectra of the seedling and the 3-month-old adult plant are rendered in a head-to-tail plot (Fig. 3), in which the top part shows the seedling spectrum and the bottom part shows the adult plant spectrum. The comparison shows that the profile of compounds observed in both cases is similar. Moreover, the observed organosulfur compounds were also detected in the positive ion mode spectrum of the unstimulated seedling root (Fig. 1A). The comparison of the negative ion mode spectra of the seedling and 3-month-old plant (both stimulated; Fig. 4) showed both similarities and differences. Most notably, several of the peaks below *m/z* 89 in the seedling spectrum were absent in the spectrum of the adult plant. These included the peaks at nominal *m/z* 46, 61, 62, 64, and 79 corresponding to thioformaldehyde, carbonate, nitrate, sulfur dioxide, methanesulfinic acid, and ethanesulfinic acid, respectively.

Mass Spectrometric Analysis of Seedling Roots Revealed the Emission of Higher Amounts of Select Organosulfur Compounds When Roots Were Stimulated

Our earlier described mass spectral analyses revealed that a cocktail of small molecules, including organosulfur volatiles, were emitted by undisturbed *M. pudica* plants even though an odor was usually not detectable by human subjects (Figs. 1 and 2). To determine the compounds responsible for the odor detected when roots were exposed to appropriate stimuli, 7-d-old unstimulated seedlings grown on agar were transferred to glass vials. For each analysis, a SPME fiber was exposed to the headspace gas produced by a single plant for 5 min, and the fiber was then analyzed by DART-HRMS in negative ion mode. Subsequently, each seedling was exposed to human skin in the manner shown in Supplemental Video S1, and the DART-HRMS analysis was repeated. The experiment was conducted in triplicate. As observed previously, the same profile of compounds found in undisturbed plants (Fig. 1) was seen, except that while the detected levels of some compounds remained constant within experimental error, the relative levels in the case of others was double, as indicated by an increase in the ion counts observed by mass spectrometry. This result is illustrated in Figure 5, which shows the difference in ion counts for compounds emitted from untouched and touched roots (depicted in blue and red, respectively). The total ion counts for the peaks at nominal *m/z* 91, 124, 165, and 198 were approximately double those observed in the unstimulated roots, ±5%. These peaks corresponded to propanesulfenic acid (*m/z* 91), 2-aminothiophenol (*m/z* 124), *S*-propyl propane-1-thiosulfinate (*m/z* 165), and phenothiazine (*m/z* 198). The identity of the compound represented by *m/z* 239 is unknown.

CS_2, Which Has Been Proposed To Be Responsible for the Smell of *M. pudica* Roots, Was Never Detected under the Soft Ambient Ionization Conditions of DART-HRMS But Only under Gas Chromatography Conditions

Despite previous reports that the odor emitted by *M. pudica* roots is caused by CS_2 (Hartel and Reeder, 1993; Piluk et al., 1998), we never detected CS_2 by DART-HRMS, even though we analyzed more than 100 seedling roots of different ages, under various growth conditions (in soil and on agar), and at different periods in the growing season (spring, summer, fall, and winter). Since CS_2 was detected previously by GC-MS, we conducted GC-MS analyses of SPME fibers exposed to *M. pudica* root volatiles for 5 min under conditions

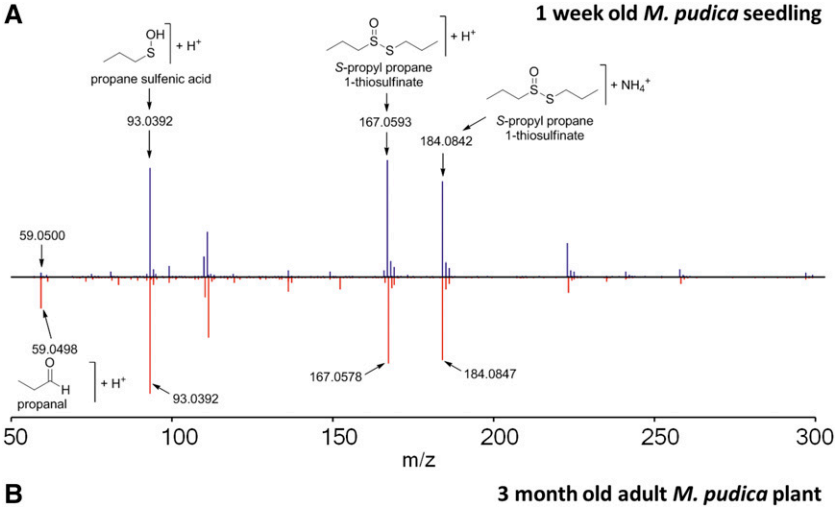

Figure 3. Head-to-tail plot of the typically observed positive ion mode DART-HRMS of the headspace gases produced by stimulated roots of 1-week-old (A) and 3-month-old (B) *M. pudica* plants. In each case, a SPME fiber was exposed to the headspace gases for 5 min, and the fiber was then analyzed by DART-HRMS. The structures shown are consistent with the high-resolution elemental compositions and isotope data obtained. Detected compounds were observed in their protonated or ammoniated form. The mass measurements and relative peak abundances associated with the data shown here are presented in Supplemental Table S3.

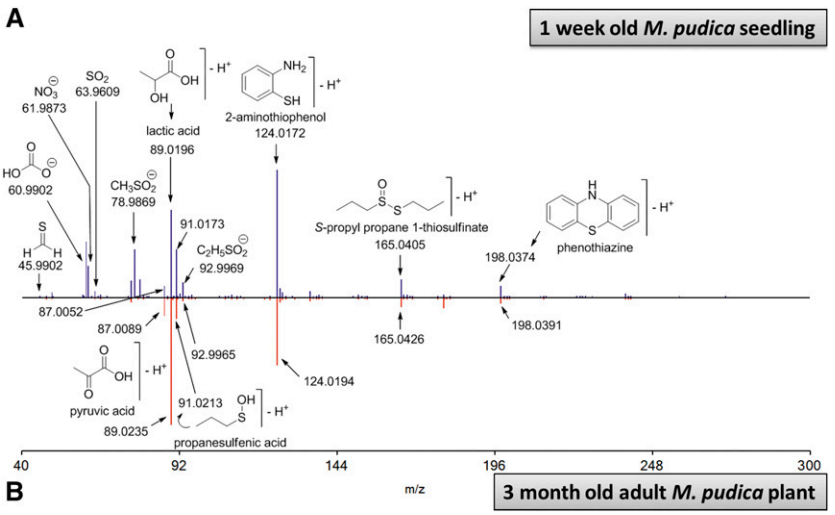

Figure 4. Head-to-tail plot of the typically observed high-resolution negative ion mode DART-HRMS of the headspace gases produced by the roots of 1-week-old (A) and 3-month-old (B) *M. pudica* plants. In each case, a SPME fiber was exposed to the headspace gases for 5 min, and the fiber was then analyzed by DART-HRMS. The structures shown are consistent with the observed high-resolution elemental compositions and isotope data obtained. Detected compounds included thioformaldehyde (nominal *m/z* 46), methanesulfinic acid (nominal *m/z* 79), and ethanesulfinic acid (nominal *m/z* 93) among other molecules. The mass measurements and relative peak intensities associated with the data shown here are presented in Supplemental Table S4.

similar to those reported previously (Piluk et al., 1998). Supplemental Figure S5 shows the GC-MS results typically observed. The gas chromatogram appears in Supplemental Figure S5A and shows that only two species, one of which was molecular oxygen, were detected. The identity of the second peak, which appeared at 1.36 min, was confirmed to be CS_2, based on the match between its electron-ionization mass spectral fragmentation pattern (Supplemental Fig. S5B) and authentic CS_2. Thus, in contrast to what was detected by DART-HRMS but consistent with previous observations, CS_2 was detected by GC-MS.

Microscopy Revealed Sac-Like Root Protuberances That Became Flattened after the Roots Were Touched with Odor-Inducing Stimuli

The observed emission of a variety of compounds from *M. pudica* roots prompted us to examine whether the roots might have structures analogous to the glandular trichomes observed on the aerial parts of plant species that secrete essential oils. Thus, we examined the roots by light microscopy. At 6× magnification, hair-like protuberances that appeared in clusters along the length of the tap root were observed (Supplemental Fig. S6).

To examine the morphology of the hair-like structures of untouched versus touched roots, 7-d-old untouched and touched seedlings that were aseptically germinated on agar were further examined by cryo-scanning electron microscopy (cSEM). Seedlings were flash frozen with liquid nitrogen just prior to analysis. Figure 6 shows representative images of unstimulated and stimulated seedling roots. On some areas of the unstimulated root, a significant number of turgid protuberances were present (Fig. 6A). A magnification of the section enclosed in a square in Figure 6A is shown in Figure 6B. Other segments of the root were only sparsely populated with protuberances, as shown in Figure 6C. Figure 6D shows an example of what was typically observed for roots that were stimulated to produce an odor. The root previously had protuberances, as observed by light microscopy (Supplemental Fig. S6), but after the root was tapped once by a human finger, the protuberances in the touched area had collapsed (Figure 6D).

Figure 7 shows a representative scanning electron microscopy (SEM) micrograph of an untouched *M. pudica* root that was acquired under cryo conditions using a microscope equipped with an EDS device for

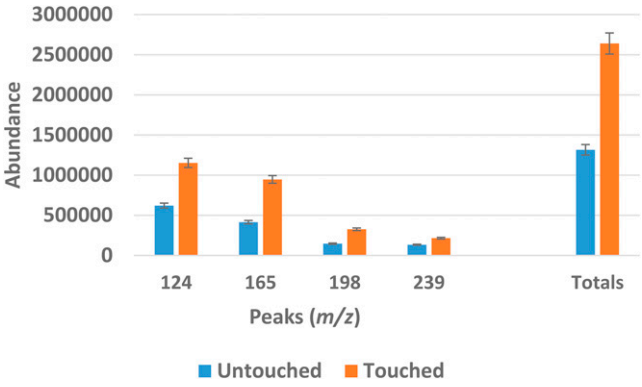

Figure 5. Differences in ion counts for some of the DART-HRMS-detected compounds emitted from untouched and touched roots (depicted in blue and red, respectively). The data represent averages of three replicates of the actual DART-HRMS-derived ion counts at each of the nominal *m/z* values shown, and the ion counts reflect the amounts of the observed ions. The *m/z* values are shown only for molecules whose touched and untouched ion counts were different within experimental error. The errors were no more than ±5% in all cases. The chemical species represented by the *m/z* values are the deprotonated form of propanesulfenic acid (*m/z* 91), 2-aminothiophenol (*m/z* 124), *S*-propyl propane-1-thiosulfinate (*m/z* 165), and phenothiazine (*m/z* 198). The identity of the molecule represented by *m/z* 239 is unknown. Totals bars represent the summation of total ion counts for all the indicated *m/z* values for the unstimulated (blue) and stimulated (red) roots.

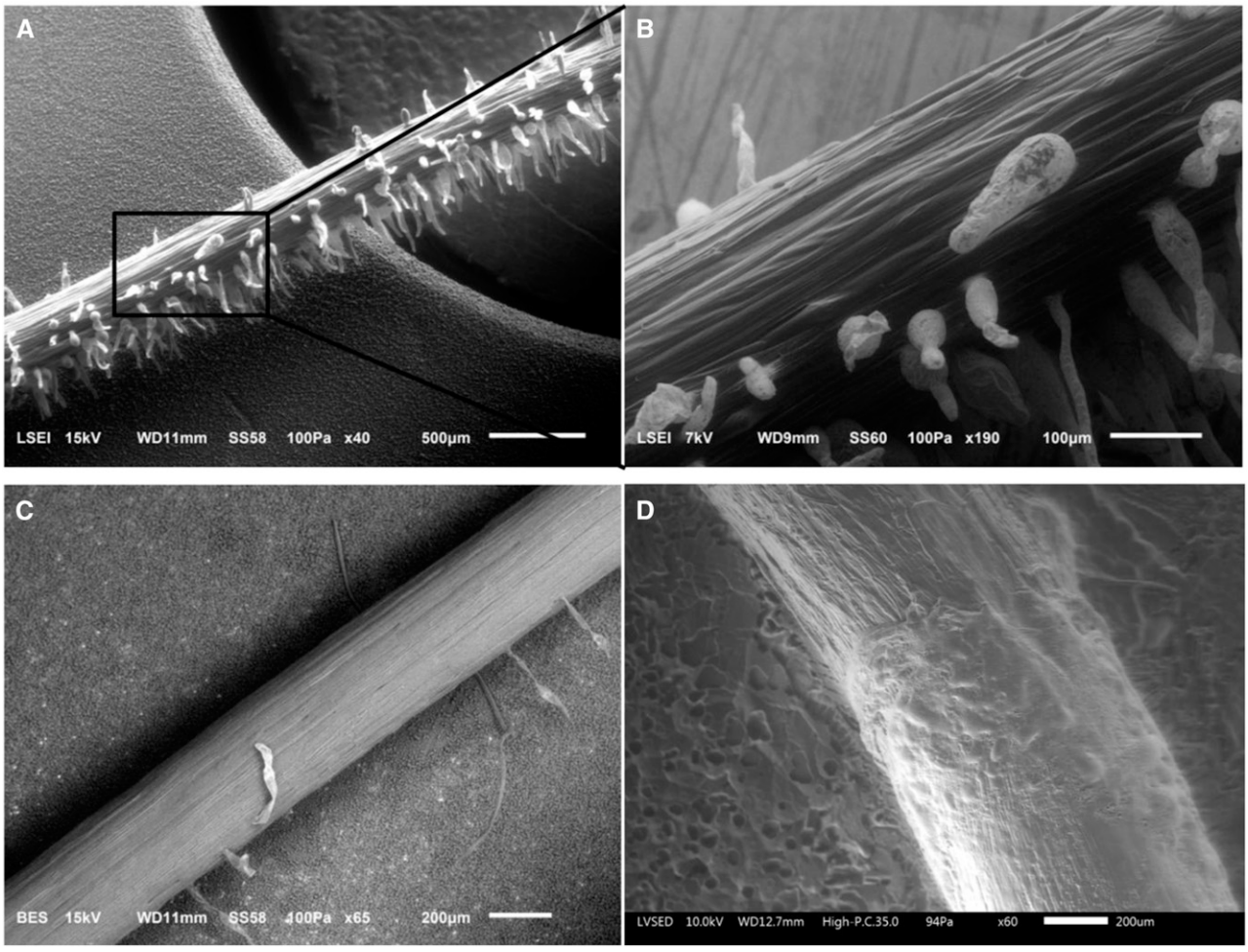

Figure 6. Representative cSEM micrographs of *M. pudica* seedling roots. A, Segment of a root showing a high density of hair-like protuberances. B, Enlargement of the boxed area shown in A. C, Segment of the same root shown in A, but distal to that appearing in A, in which the population of protuberances is sparse. Observed protuberances were 100 to 200 μm in length. D, Touched segment of a root shaft that was shown previously by light microscopy to have protuberances. The protuberances are no longer present. The scale bar represents 200 μm.

elemental analysis determination. The cSEM micrograph is shown in Figure 7A. Each of the elements detected in the x-ray map is represented by a different color (Fig. 7B). The hue of the micrograph of the root segment shown in Figure 7A reflects the composite of the overlaid color-coded contributions of the elements detected. The map sum spectrum of the elements detected and their relative amounts are shown in Figure 7C. The EDS analysis revealed that, besides the expected carbon, nitrogen, and oxygen contributions expected to be present in living tissue, other elements detected included potassium, chlorine, calcium, sulfur, phosphorus, and magnesium at 13.1, 2.6, 1.7, 1.4, 0.5, and 0.4 weight %, respectively (Fig. 7C). The amounts of K^+ and Cl^- were significant enough in some of the protuberances that an outline reflecting the presence and topography of the hairs in the cSEM image shown in Figure 7A can be seen in the K^+ and Cl^- maps (Fig. 7B). The microscopic protuberances, which were flattened under the high-vacuum conditions of the experiment, varied in length between 100 and 200 μm and had a sac-like appearance, with several having relatively high localized levels of K^+ and Cl^-, as revealed by EDS.

Figure 8 (top) shows the cSEM micrograph of a root segment on a bed of agar whose left side was exposed to human skin and whose right side was untouched. The sacs that were previously on the left side of the root (as observed by light microscopy) had collapsed, consistent with our previous observations (Fig. 6D). However, sacs still appeared on the right side (untouched) of the root segment. EDS analysis was performed on the three sections of the root labeled spectrum 1, spectrum 2, and spectrum 3 in Figure 8 (top) in order to assess the similarity of the elemental profile of stimulated versus unstimulated root sections. The EDS map sum spectra illustrating the elemental compositions for the three sections are shown at the bottom of Figure 8. Comparison of the three spectra from the three root areas

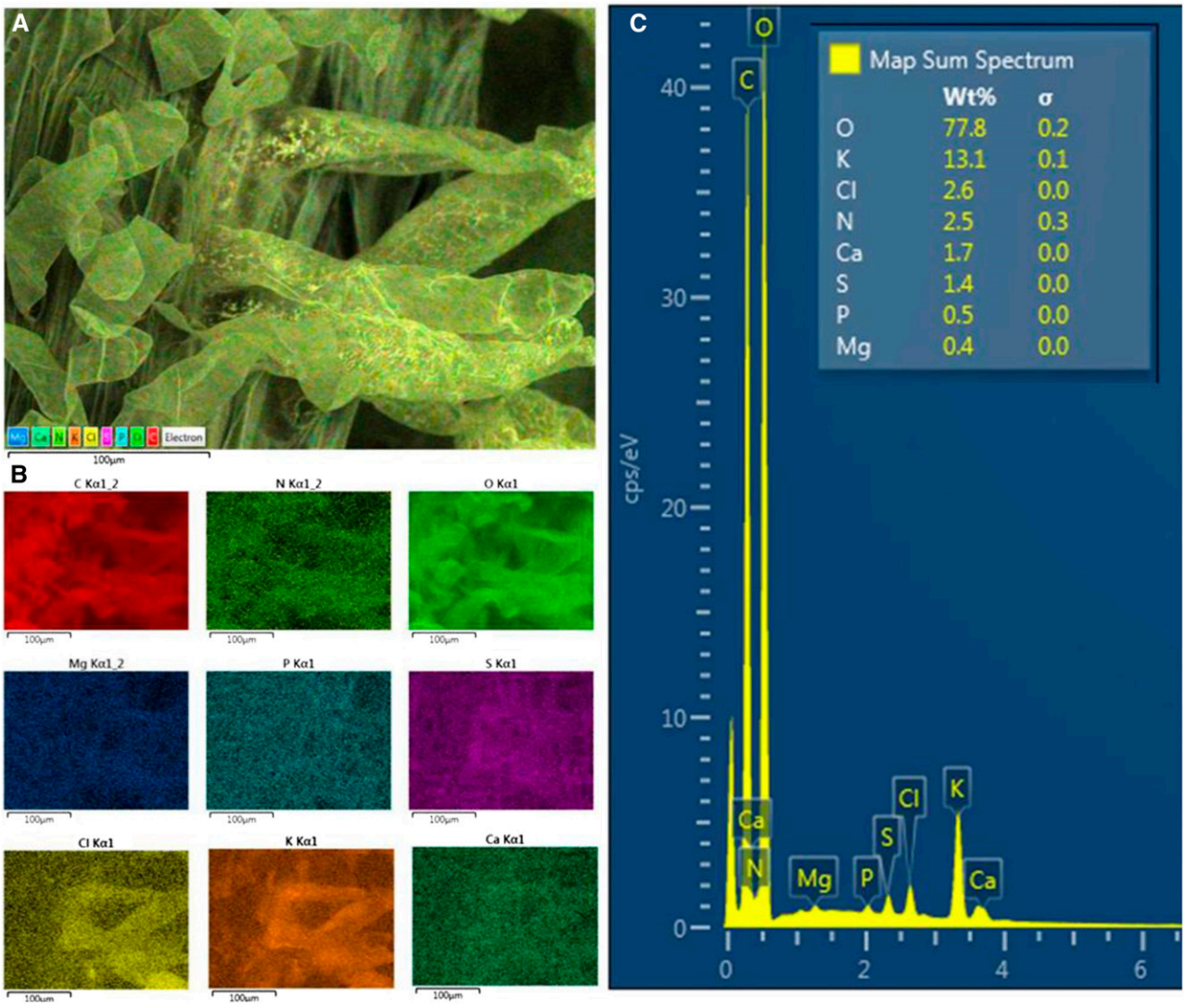

Figure 7. Representative cSEM EDS micrograph of a section of an *M. pudica* seedling root densely populated with hairs that are flattened (as opposed to turgid) under the high-vacuum conditions of the analysis. A, The hue of the image reflects the composite of the overlaid color-coded contributions of the elements carbon (C), nitrogen (N), oxygen (O), magnesium (Mg), phosphorus (P), sulfur (S), Cl^-, K^+, and Ca^{2+}. B, X-ray maps of each of the color-coded elements contributing to the color composite shown in A. Whereas, in some cases, such as for carbon, nitrogen, and oxygen, there is uniform elemental distribution, the concentrations of Cl^- and K^+ are significant enough in some of the hairs that a general outline reflecting the topology of those hairs in the cSEM image is revealed in their maps. C, Elemental composition map sum spectrum of the cSEM image shown in A. The relative percentage contributions by weight % are listed and show that, besides carbon, nitrogen, and oxygen, K^+ and Cl^- are present at the highest relative concentrations.

sampled showed that, although the level of K^+ was similar for the spectrum 1 and 2 areas (i.e. 6 ± 0.1 and 5.2 ± 0.1 weight %, respectively), that in the spectrum 3 area (which was farthest away from the area that was touched) was almost double, at 10.8 ± 0.1 weight %. Similar trends were observed for Cl^-, Ca^{2+}, and sulfur. For the spectrum 1 and 2 sampled areas, which were close to the part of the root that was stimulated by exposure to human skin, the Cl^- levels were 0.8 ± 0.1 and 0.7 ± 0.1 weight %, respectively, whereas a Cl^- level of 2.3 ± 0.1 weight % was observed in the spectrum 3 area. For Ca^{2+}, the relative amounts observed for the spectrum 1, 2, and 3 areas of the root segment were 1.1 ± 0.1, 1.2 ± 0.1, and 2.3 ± 0.1 weight %, respectively, showing that the amount of Ca^{2+} in the sample 3 area was double that observed in the spectrum 1 and 2 areas. The amount of sulfur in the spectrum 3 area was 1.4 ± 0.1 weight %, whereas that for the spectrum 1 and 2 areas was 0.9 ± 0.1 and 1 ± 0.1 weight %, respectively, showing that the amount of sulfur in areas 1 and 2 was similar, while that in area 3 was higher. Quantitation (i.e. determination of the actual amounts of the

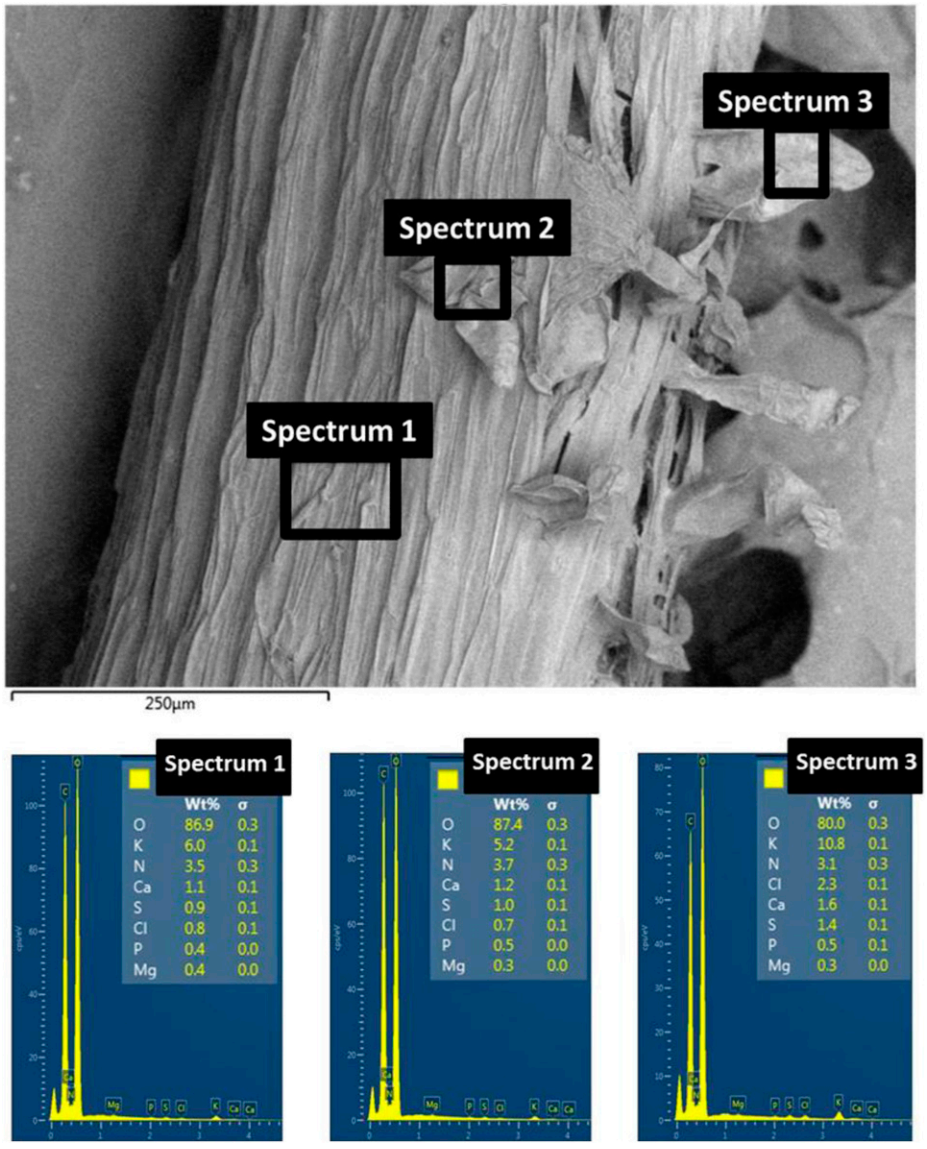

Figure 8. cSEM micrograph with EDS analysis of a section of an *M. pudica* root the left side of which had been touched with a finger. The root sample was flash frozen at liquid N_2 temperature immediately after an odor was detected. The cSEM micrograph (top) shows an *M. pudica* root section that, prior to being touched, was shown by optical microscopy to be heavily populated with protuberances on both sides. The micrograph shows that, consistent with previous observations, the protuberances on the touched side of the root were no longer present. A few flattened sacs can be seen on the right side. The EDS spectra for the indicated boxed inspection fields shown in the micrograph are seen in blue (bottom) with the observed elements, as described in Figure 7, indicated by relative weight % with SDS shown.

elements in stimulated versus unstimulated roots) could not be made because quantitation by EDS requires that the sample be (1) perfectly flat, (2) homogenous, and (3) infinitely thick to the x-ray beam. Since plant roots do not fit these criteria, the actual amounts of the elements could not be determined. Attempts were also made to perform quantitation using x-ray fluorescence. However, these efforts were unsuccessful, because the sample handling required to conduct the experiment always resulted in the emission of volatiles. Thus, it was not possible to acquire before-touch and after-touch results that could be compared for different samples. However, in order to confirm the reproducibility of the results, the experiment was repeated several times, and in all cases, the same aforementioned trends were observed. Thus, another example is shown in Supplemental Figure S7. The segment of the root shown above the line in the cSEM micrograph is the untouched portion, while that below the line was touched with a finger. The sections labeled 1, 2, and 3 in the micrograph are those areas that were analyzed by EDS, and the EDS results are shown beneath the cSEM micrograph and labeled spectrum 1, 2, and 3, respectively. Similar to the results presented in Figure 8, the section of the root farthest from the touched area exhibited the highest levels of K^+ and Cl^- (5.7 and 0.8 weight %, respectively), while the relative levels of these ions for the touched area were 2.6 and 0.5 weight %, respectively).

DISCUSSION

In this article, we describe our observation of four, to our knowledge, heretofore unreported phenomena: (1) the emission of compounds from roots in response to a

touch stimulus; (2) the ability of the root to distinguish between different types of stimuli, such as responding to exposure to soil or the touch of a finger but not to other stimuli; (3) the emission and detection of highly reactive and elusive organosulfur intermediates, including thioformaldehyde, in addition to other unique species; and (4) the presence of sac-like microscopic protuberances along *M. pudica* root shafts.

The finding that *M. pudica* roots secrete increased levels of metabolites in response to touch is particularly remarkable in light of the fact that the aerial parts of the plant are also touch sensitive. The sac-like protrusions, which were revealed by light microscopy and cSEM to appear in clusters along the root shaft, are reminiscent of the well-known glandular trichomes that have been observed on the aerial parts of many plants and that manufacture and emit a diversity of secondary metabolites (Tissier, 2012). Root hairs with glandular morphologies that secrete small-molecule organics have been observed in sorghum (*Sorghum bicolor*; Netzly and Butler, 1986) and apple (*Malus domestica*; Head, 1964) seedlings. However, those that appear in *M. pudica* may be most analogous to the exploding glandular trichomes seen on aerial parts of *Sicana odorifera* (Kellogg et al., 2002) and *Salvia blepharophylla* (Bisio et al., 1999) and proposed to have been present in the extinct seed fern *Blanzyopteris praedentata* (Krings, 2002; Krings et al., 2003) that release exudate in response to touch.

Plant root tip cells exhibit a form of responsiveness to touch whereby they can circumvent barriers encountered in soil that obstruct their downward trajectory. For example, in Arabidopsis (*Arabidopsis thaliana*), the gravitropism normally displayed by plant roots is supplanted with a thigmotrophic response when the downward direction of growth is impeded by a barrier (Okada and Shimura, 1990; Massa and Gilroy, 2003). However, the ability of roots to distinguish between types of stimuli was surprising and, to our knowledge, is not a previously reported phenomenon. Nevertheless, this behavior seems analogous to a characteristic of the aerial parts of plants that exhibit mechanostimulatory activity. It was noted by Darwin (1880, 1893), for example, that although the carnivorous response of *Drosera rotundifolia* is induced by contact between insect prey and the plant's tentacles, these same tentacles do not respond to rain or wind. Some flowers are also known to explosively release pollen in response to touch. For instance, male flowers of the orchid *Catesetum saccatum* forcefully release their pollen sacs in response to touch by an insect of the antennae at the center of the flower. How the plants distinguish between the different forms of stimuli (e.g. insect versus inanimate object) is not fully understood, and we do not yet know the mechanism by which *M. pudica* emits small molecules in response to various stimuli. Interestingly, although a single tap by a finger of an *M. pudica* root reliably resulted in odor emission, the same was not true of other odor-eliciting stimuli. For example, exposing a root to soil by gently tapping it once on the soil surface did not produce an odor, whereas dragging the root across the surface (as shown in Supplemental Video S2) reliably produced a strong odor. Although the latter observation implied that odor emission was a consequence of rupturing of the sacs that appeared along the root shaft, this conclusion did not explain why a single tap on the root by a human finger produced an odor but a similar action with glass did not. Additional, more extensive studies are being conducted to investigate the mechanism of this phenomenon.

The composite of small-molecule species detected by high-resolution positive and negative ion mode DART-HRMS provided an unprecedented glimpse of the in situ root emissions and further expands on the recently demonstrated utility of ambient ionization mass spectrometry techniques in the detection of plant-derived organosulfur volatiles (Domin and Cody, 2014). These include the demonstrations (Block et al., 2010, 2011; Kubec et al., 2010) that various organosulfur intermediates that are formed when the tissues of onion, garlic (*Allium sativum*), *Allium siculum*, and *Petiveria alliacea* are injured can be detected in real time by DART-HRMS. Of particular relevance is the finding that the changing profile of organosulfur exudates that occurs in *Brassica* spp. roots in response to herbivore attack or a tissue breach can be monitored in real time by proton transfer reaction-mass spectrometry (Crespo et al., 2012; Danner et al., 2012; van Dam et al., 2012; Samudrala et al., 2015). If conventional metabolome analysis sample preparation methods had been used in these cases (e.g. plant tissue disruption followed by solvent extraction and GC- or LC-MS analysis of the extract), it would not have been possible to distinguish between compounds emitted into the environment by the roots and those that were intracellular. Furthermore, the solvent extraction step used in many conventional analysis methods selects for the subset of compounds that are most well solubilized in the solvent used; thus, not all compounds present are detected. These factors underscore the utility of these ambient ionization mass spectrometry techniques as tools for the investigation of in situ plant emissions in a manner that does not interfere with the biological processes of the system.

In order to confirm that organosulfur volatile contributions were from the roots and not the plant's aerial parts, a small growth chamber was designed in which a plug of agar separated the aerial parts from the roots. When placed on the bed of agar, the seedling tap root grew through the agar and emerged on the opposite side. This construct permitted independent analysis of both the roots and aerial parts without disturbing the plant or disrupting the plant tissue. Furthermore, as the experiment was conducted under sterile conditions, there were no microbe-derived contributions to the headspace volatiles. Using this method, we were able to confirm that the aerial parts did not contribute detectable organosulfur volatiles.

As compared with hydrocarbons, organooxygen, and organonitrogen compounds, organosulfur molecules are well known to have low odor thresholds (nanoliters per liter for organosulfur compounds versus microliters per liter for organooxygen and organonitrogen compounds;

Leonardos et al., 1969). Therefore, we were surprised by the observation that plant roots emitted organosulfur volatiles that were detectable by DART-HRMS in the absence of a stimulus, even though they were not detectable to humans by smell. Since mass spectrometric analysis showed that human olfactory detection was associated with an apparent doubling of the emission of a subset of root volatiles, we conclude that emissions from nonstimulated roots were at levels below the nanoliters per liter olfactory threshold for the panelists in our study. It should be noted that the use of SPME fibers to sample headspace gases served to concentrate the volatiles, which means that the levels in the headspace of compounds detected by SPME analysis were much lower than were implied by our ability to detect their presence on the fiber. Our observations also raise the possibility that there may have been some odor compounds that went undetected by the form of analysis used in this study. In our experiments, PDMS SPME fibers were used to concentrate the headspace gases so that their constituents would be at high enough levels to be detected. The fibers were exposed to the headspace for 5 min (as opposed to 30 min, which is used when one wishes to saturate the fibers) in order to be able to differentiate between the relative levels of emitted compounds. Thus, one way in which to determine whether additional odor compounds were present would have been to extend the exposure time of the SPME fiber to the headspace in order to capture the maximum range and levels of compounds possible. We conducted this experiment by exposing PDMS SPME fibers to the headspace of numerous *M. pudica* roots (stimulated and unstimulated) for 30 min. Subsequent DART-HRMS analysis revealed chemical profiles identical to those obtained for stimulated and unstimulated roots that had been exposed to PDMS fibers for 5 min (data not shown). This result supports the premise that we detected most if not all of the analytes that were present. However, it is also possible that there may have been odor compounds present that were not adsorbed to the PDMS fiber. Thus, we have concluded that, at a minimum, there were five compounds represented by nominal *m/z* 91, 124, 165, 198, and 239, whose increased emission from *M. pudica* roots in response to appropriate stimuli was correlated with odor detection by human subjects.

Although odiferous organosulfur compounds featured heavily in this mix of emitted molecules, noticeably absent was the CS_2 reported by Piluk et al. (1998). Published studies on the analysis of CS_2 production in Mimosoideae species are similar in that they have all involved (1) detection of CS_2 after root tissue disruption; (2) a significant time delay between tissue disruption and CS_2 analysis; and (3) detection of CS_2 under high GC injector temperature conditions (100°C–250°C; Haines, 1991; Hartel and Reeder, 1993; Feng and Hartel, 1996; Piluk et al., 1998), a factor known to result in rapid and facile degradation of labile organosulfur compounds (Block, 2011). The fact that optimal CS_2 production has been observed only after tissue disruption and a significant delay between tissue rupture and analysis time could mean that the chemistry resulting in the appearance of CS_2 was subsequent to earlier stage reactions that rapidly produced compounds that served as a first line of chemical defense and that were later degraded to CS_2. Additionally, the GC conditions used for the analysis of organosulfur compounds are notorious for promoting reactions in the gas chromatograph injection port that result in the production of compound artifacts (Block, 2011). In light of this finding and our own observations outlined herein, it is possible that the CS_2 reported previously is not produced by the plant per se but rather is formed from precursors that, under the GC conditions used, degraded to form CS_2. This hypothesis is supported by our observation that, in contrast to the diversity of compounds detected by DART-HRMS analysis of SPME fibers exposed to *M. pudica* root volatiles, GC-MS analysis under published conditions as well as gas chromatography analysis of PDMS SPME fibers that had been exposed to the headspace were the only conditions under which CS_2 was observed (Supplemental Fig. S5). This implies that these compounds, when observed previously by GC-MS, were artifacts of the experimental protocol used for their detection (Haines et al., 1989; Farkas et al., 1992; Hartel and Reeder, 1993; Feng and Hartel, 1996; Piluk et al., 1998).

Several of the compounds emitted by *M. pudica* roots are consistent with those that would be expected from Cys lyase-mediated degradation of djenkolic acid, a

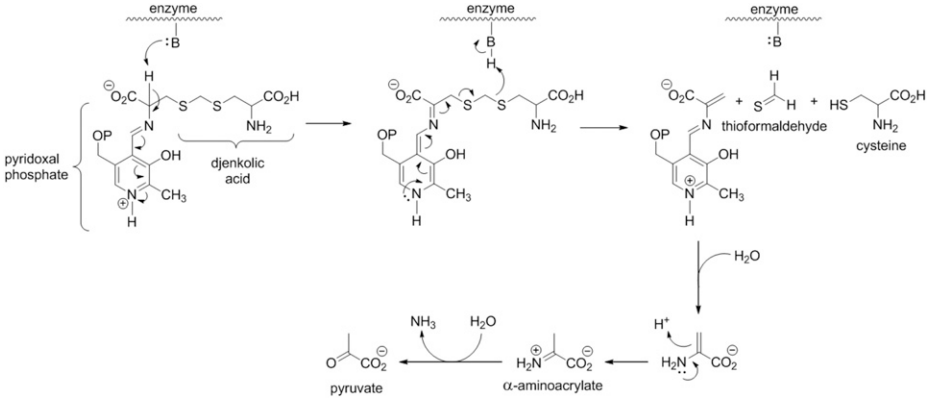

Figure 9. Proposed mechanism for Cys lyase-mediated degradation of djenkolic acid. In the first step, a Schiff base forms between djenkolic acid and the enzyme-derived pyridoxal phosphate. Enzyme-promoted proton abstraction from an α-carbon in the djenkolic acid-pyridoxal phosphate complex ultimately results in the liberation of thioformaldehyde, Cys, and a pyridinium ion, hydrolysis of which yields α-aminoacrylate. Further hydrolysis of this intermediate furnishes ammonia and pyruvate.

compound detected in *M. pudica* roots (Piluk et al., 1998). A putative mechanism for the formation of these volatiles from djenkolic acid is shown in Figure 9, and it accounts for the observation of thioformaldehyde, pyruvate, and ammonia. Thioformaldehyde, a fleeting unstable species under ambient conditions (Solouki et al., 1976), is a constituent of interstellar clouds (Agúndez et al., 2008). It has been formed by thermolysis, photolysis, or vacuum pyrolysis of appropriate precursors, and it has been observed by microwave spectroscopy (Penn et al., 1978) or trapped in low-temperature matrices for structural studies (Jacox and Milligan, 1975; Solouki et al., 1976; Torres et al., 1982; Watanabe et al., 1991; Suzuki et al., 2007). Its detection (albeit in trace amounts), like that of the sulfenic and sulfinic acids observed here and in recent studies of *Allium* spp. by Block et al. (2010), is quite remarkable and speaks to the utility of DART-HRMS in the characterization of reactive organosulfur intermediates.

The mechanism by which the roots are responsive to touch is unclear. However, the observations that untouched root hairs contain relatively high levels of K^+ and Cl^- (Fig. 8; Supplemental Fig. S7) and that touched root segments have lower relative levels of K^+ and Cl^- compared with untouched sections of the same root (Fig. 8; Supplemental Fig. S7) may indicate that the process is similar in some ways to that which has been proposed to cause movement in the aerial parts of the plant. *M. pudica* leaf closing in response to touch is controlled by specialized structures called pulvini that appear at the base of the petioles. Movement occurs when cells within the pulvini lose water and turgor, which has been proposed to be triggered in part by the transport of K^+ and Cl^- ions in pulvini cells (Simons, 1981; Fromm and Eschrich, 1988; Visnovitz et al., 2007; Volkov et al., 2010a, 2010b, 2014).

The seismonasty exhibited by the aerial parts of *M. pudica* has been suggested to be a defensive strategy whose suddenness may serve to scare or shake off intruders (Pickard, 1973), give the appearance of a less voluminous meal (Braam, 2005), or make more apparent to would-be predators the menacing thorns sported by the plant stems (Eisner, 1981). However, the purpose of the mechanostimulatory behavior of the roots and the role of the compounds emitted are not immediately apparent. Given the inherent complexities of rhizosphere ecosystem biology, further systematic studies will be necessary to determine the functions of the root protuberances and the small-molecule emissions. These are areas of continuing study in our laboratories.

MATERIALS AND METHODS

Plants

Mimosa pudica seeds were obtained from Seedvendor.com. They were immersed in 70% (v/v) aqueous ethanol for 1 min, rinsed with sterile water, submerged in 3.075% sodium hypochlorite (50% [v/v] solution of Clorox) containing 0.05% (v/v) Tween 20 for 10 min, and rinsed nine times with 23°C sterile water. Seeds were placed in 70°C sterile water for 16 h at 23°C. Using sterile tweezers, five to six seeds were placed on 100- × 15-mm or 150- × 15-mm petri dishes containing 1× Murashige and Skoog medium with vitamins (PhytoTechnology Laboratories) and 44 mM Suc solidified with 2% (w/v) tissue culture-grade purified agar (PhytoTechnology Laboratories). Seeds germinated within 2 to 3 d and were grown under fluorescent lights with 16 h of light per day at 23°C. *M. pudica* seeds germinated in soil were first scarified by suspending them in 70°C deionized water for 16 h at 23°C. Using tweezers, three to four seeds were placed within each receptacle in a 36-cell greenhouse kit according to the manufacturer's specifications (Burpee). Germination occurred within 4 d. Seedlings were transplanted 14 d after germination into Miracle-Gro flower and vegetable garden soil in 6-inch pots under greenhouse conditions. Plants were watered daily.

Headspace SPME Sampling

A 2-cm, 50/30-μm divinylbenzene/carboxen/polydimethylsiloxane 24-gauge Stableflex fiber (Sigma-Aldrich), mounted within a manual SPME fiber holder assembly (Sigma-Aldrich), was used for the analysis of headspace gases. SPME fibers were conditioned by heating at 250°C in a helium gas stream for 2 h just prior to analysis and were subjected to mass spectrometric analysis to confirm the absence of adsorbed species prior to sampling of headspace gases. For seedling analysis, 1-week-old plants that were aseptically germinated on the agar surface were gently lifted at the stem just beneath the cotyledons and immediately placed in a 15-mL clear glass vial (o.d. × height × i.d., 21 mm × 70 mm × 12 mm; thread 18-400; Sigma-Aldrich), which was capped with a Mininert screw-thread valve (Sigma-Aldrich). For root stimulation experiments, the seedling root was touched with a finger as shown in Supplemental Video S1 prior to placing it in the vial. The process of touching the root and depositing it into the vial took approximately 10 to 15 s. The manual SPME fiber assembly equipped with a conditioned SPME fiber was then inserted into the valve of the Mininert cap, and the fiber was exposed to the headspace gases for 5 min at 25°C. Mass spectrometric analysis of the fiber was then conducted either by DART-HRMS or GC-MS. The headspace gases of adult plants were sampled similarly. The entire potted plant was placed into a jar (1.88 L, 12-cm i.d., 21 cm in height), which was sealed with an air-tight cap that was outfitted with a rubber septum through with the SPME fiber assembly was inserted. After exposure to headspace volatiles for 5 min, the SPME fiber was retracted, the fiber assembly was removed, and the fiber was then immediately subjected to mass spectrometry analysis. Adult plant root stimulation experiments were conducted similarly, except that the plant to be analyzed was uprooted from the soil, the bulk of the soil was gently removed, and the entire plant was deposited within the 1.88-L jar as described above.

Separation of the *M. pudica* Aerial and Root Parts for Independent Headspace Sampling

An apparatus composed of a Pyrex glass rod (25.4 mm o.d.) and a Pyrex cylindrical tube (26.4 mm i.d., 30 mm o.d.; both purchased from Sci-Tech Glassblowing) was created (Supplemental Fig. S3). Both the glass rod and tube were cut into 90-mm sections. An O-ring (7/8 × 1 inch) was placed on the middle of the rod. The rod was inserted into the cylindrical tube, and the O-ring served to allow the rod to reach only halfway into the tube. The opposite open end of the tube was covered with foil, and the entire setup was sterilized. Subsequently, approximately 5.5 mL of plant medium described above, composed of Murashige and Skoog medium with vitamins (PhytoTechnology Laboratories), Suc, and plant cell culture-tested agar (Sigma-Aldrich), was poured into the open end of the cylindrical tube. After it had solidified, the glass rod was removed from the opposite end of the tube, leaving behind a 1-mm-thick disc of agar. One end of the tube was sealed with a sterile rubber sleeve septum (12.7 mm bottom i.d., 23.7 mm o.d.; Sigma-Aldrich). An aseptically germinated 3-d-old *M. pudica* seeding was placed on the agar surface using sterile tweezers. Sterile water (20 mL) was injected through the bottom septum, and the open end of the tube was lightly covered with sterilized Parafilm to prevent the agar from drying out. Within 48 h, the seedling root had emerged from the opposite side of the agar disc, such that the agar served to completely separate the headspace of the aerial and root parts. To sample the root headspace, the water was withdrawn via syringe and the PDMS SPME fiber was inserted into the septum. For sampling of the aerial headspace, a rubber septum was applied to the top of the tube and the PDMS SPME fiber was inserted into the septum. Sampling and analysis occurred as described above.

Mass Spectrometric Analysis

An AccuTOF-DART (JEOL USA) high-resolution time-of-flight mass spectrometer was used for mass measurements. The instrument and experimental

conditions for the direct analysis in real time time-of-flight mass spectrometry measurements were performed as described previously (Kubec et al., 2010), except that headspace gases were first adsorbed onto a SPME fiber, which was then analyzed at 250°C. For analysis, the fiber was held for a few seconds at the mass spectrometer inlet, and the resulting spectrum was recorded until no desorbed molecules were detected. Calibration, spectral averaging, background subtraction, and peak centroiding of the mass spectra were performed using TSSPro3 (Shrader Software Solutions) data processing software. Mass Mountaineer software (www.mass-spec-software.com) was used for mass spectrum analyses, spectral elemental composition, and isotope analyses. Calibration was performed using a polyethylene glycol mixture (PEG 200, 400, 600, and 1000). Experiments in which changes in the emission profiles of molecules were monitored (to compare unstimulated and stimulated roots) were performed in negative ion mode. The experiments were conducted in triplicate. The *m/z* values for molecules whose unstimulated versus stimulated ion counts were different within experimental error were selected in TSSPro and subjected to peak area integration for each SPME fiber analysis. Reconstructed ion chromatograms of these peaks for each sample were exported to Excel. The total peak area counts for the individual *m/z* values were calculated for each sample and then summed to get the overall peak area counts. The three replicate individual peak area counts were averaged, and the average overall peak area count was calculated. GC-MS analysis was conducted using an Agilent HP 6890 gas chromatograph coupled to an HP 5972A mass selective detector (Agilent Technologies). Headspace gases from root-stimulated plants were sampled and analyzed as described previously (Haines, 1991) using a capillary column (HP-5 mass spectrometer, 30 m × 0.25 mm, 0.25 μm) under the following conditions: oven temperature, 50°C, raised linearly at a rate of 20°C min^{-1} to 200°C; inlet temperature, 100°C; inlet mode, splitless; carrier gas, helium, with a flow rate of 1 mL min^{-1}; ionization mode, EI^+, 70 eV, and 300 μA.

Microscopy

Scanning electron microscopy imaging of untouched and touched seedlings was done under cSEM at liquid N_2 temperature. Two methods were used.

Method 1

A 1-week-old seedling was carefully placed onto an SEM sampling block (JEOL USA) that was outfitted with two clamps that were used to hold the seedling in place. The entire setup was then plunged into a Dewar of liquid N_2, where it was allowed to equilibrate. The sampling block with the seedling was then viewed with a JSM-6610LV scanning electron microscope (JEOL USA). With the samples prepared in this way, the turgor of the roots was maintained for a significant period during the analysis (as illustrated in Fig. 4).

Method 2

An SEM sampling block (JEOL USA) was immersed in liquid N_2 for 15 min. The block was then removed from the liquid N_2, and a 1-week old seedling was contact frozen by quickly placing it onto the liquid N_2-cooled SEM sampling block. The sample was then imaged using a JSM-IT300LV scanning electron microscope (JEOL USA).

Light Microscopy

M. pudica roots were viewed using a Nikon stereozoom SMZ800 microscope that was equipped with a Nikon DS Fi2 microscope camera.

X-Ray Fluorescence

X-ray fluorescence measurements were made with a JEOL USA JSX-1000 benchtop energy-dispersive x-ray fluorescence spectrometer.

Root Stimulation Experiments

The roots of *M. pudica* seedlings that were germinated aseptically on agar were lifted from the agar bed with stainless steel tweezers at the stem beneath the cotyledon and exposed to human skin and soil as shown in Supplemental Videos S1 and S2, respectively. To determine whether exposure to other forms of matter elicited an odor detectable to humans, roots were touched with the following materials, either by a single tap with the material, as shown in Supplemental Video S1, or, in the case of soil, by dragging the root across the surface, as shown in Supplemental Video S2: a 12- × 0.2-inch metal spatula (410 stainless steel; Fisher Scientific); a 6- × 0.19-inch glass stirring rod (Fisher Scientific); and a 4-inch wooden toothpick (Diamond L'Elegance extra long toothpicks, no additives). For some experiments, exposure of roots to the metal, glass, and wood stimuli was performed while the roots were being viewed using a Nikon stereozoom SMZ800 microscope in order to determine whether the structures along the root shaft were modified on exposure to the various materials. For other experiments, roots were imaged by cSEM both before and after exposure to human skin.

Odor Detection

Odor emission from 7-d-old *M. pudica* seedlings was assessed by a panel of five individuals who evaluated the samples as either having no detectable odor or a detectable odor. Each panelist was exposed to five seedlings before and after stimulation. Seedlings were suspended approximately 1 inch from the nose of each panelist before and after root stimulation.

Odor Emission Experiments

Odor emission from 7-d-old *M. pudica* seedlings could be elicited by dragging seedling roots across the surface of soil or subjecting the seedling to a single tap by a human finger (as shown in Supplemental Videos S2 and S1, respectively). For the soil experiments, 30 g of Miracle-Gro garden soil was dispensed into a petri dish bottom (100- × 25-mm polystyrene dish; PhytoTechnology Laboratories). One-week-old *M. pudica* seedlings were carefully lifted from agar plates at the seedling stem just beneath the cotyledon with stainless steel tweezers. Seedling roots were then dragged along the soil surface while being held with the tweezers (Supplemental Video S2). For the human finger-touch experiments, 7-d-old *M. pudica* seedling roots were tapped once with a finger as shown in Supplemental Video S1. To test whether an odor could be detected if the seedling root was exposed to other forms of matter, seedling roots were tapped once with (1) a 6- × 0.19-inch glass stirring rod (Fisher Scientific); (2) a 12- × 0.2-inch metal spatula (410 stainless steel; Fisher Scientific); and (3) a 4-inch wooden toothpick (Diamond L'Elegance extra-long toothpicks, no additives). The influence of stimulation of the aerial plant parts on the detection of an odor was also determined. The cotyledons of 7-d-old seedlings whose roots had not been exposed to odor emission stimuli were held between the thumb and forefinger for 5 to 30 s and released. Whether an odor was detected was then recorded.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. *M. pudica* seedlings germinated on agar showing the single tap root that emerges.

Supplemental Figure S2. Representative headspace gas analysis assembly used to sample the gases produced by agitated *M. pudica* seedling roots.

Supplemental Figure S3. Glass growth chamber apparatus designed to sample and detect the headspace gases of root versus aerial parts of *M. pudica* seedlings independently.

Supplemental Figure S4. Determination of odor emission in stimulated and unstimulated roots by a five-person untrained human panel.

Supplemental Figure S5. Typical results obtained for the GC-MS analysis of the headspace of *M. pudica* roots.

Supplemental Figure S6. Light microscopy image of a portion of an *M. pudica* seedling root at 6× magnification, showing hair-like structures that appeared in clusters along the root shaft.

Supplemental Figure S7. cSEM micrograph with EDS analysis of a section of an *M. pudica* root.

Supplemental Table S1. Mass measurements for the positive- and negative-ion mode DART-HRMS spectra of the headspace of a 7-d-old *M. pudica* seedling in the absence of an odor-producing stimulus.

Supplemental Table S2. Mass measurements for the negative-ion mode DART-HRMS spectra of root and aerial parts of a 1-week-old *M. pudica* seedling.

Supplemental Table S3. Mass measurements for the positive-ion mode DART-HRMS spectra of *M. pudica* seedling and adult plants after root stimulation.

Supplemental Table S4. High-resolution mass measurements for the negative-ion mode DART-HRMS spectra of agitated *M. pudica* seedling and adult plant roots.

Supplemental Video S1. Demonstration of how to elicit emission of odor compounds from an *M. pudica* root by exposure of the root to human skin.

Supplemental Video S2. Demonstration of how to elicit emission of odor compounds from an *M. pudica* root by exposure of the root to soil.

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