Article Addendum

Impact of (±)-catechin on soil microbial communities

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Catechin is a highly studied but controversial allelochemical reported as a component of the root exudates of Centaurea maculosa. Initial reports of high and consistent exudation rates and soil concentrations have been shown to be highly inaccurate, but the chemical has been found in root exudates at and much less frequently in soil but sporadically at high concentrations. Part of the problem of detection and measuring phytotoxicity in natural soils may be due to the confounding effect of soil microbes, and little is known about interactions between catechin and soil microbes. Here we tested the effect of catechin on soil microbial communities and the feedback of these effects to two plant species. We found that catechin inhibits microbial activity in the soil we tested, and by doing so appears to promote plant growth in the microbe-free environment. This is in striking contrast to other in vitro studies, emphasizing the highly conditional effects of the chemical and suggesting that the phytotoxic effects of catechin may be exerted through the microbes in some soils.

Centaurea maculosa, a Eurasian invader in North America, has been reported to release (±)-catechin (hereafter referred as catechin) from its roots, and this chemical has been shown to be phytotoxic in vitro, in sand cultures and in the field. A number of other authors have reported phytotoxicity of the (+)-catechin form and the (-)-catechin form has been reported to inhibit green algae. However, other authors have found no phytotoxic effects of forms of catechin or phytotoxicity only at unreasonably high concentrations. Perry et al., using a minimum detection limit of 25 μg g⁻¹, detected catechin in only 20 soil samples out of 402, but this was for a set of plants repeatedly measured over a season, and at one point in time all plants at the site were associated with catechin in the soil at a very high mean concentration (650 ± 450 (SD) μg g⁻¹). Extreme variation in detection may be due to pulsed releases, but catechin is a very insensitive species to the chemical and was manifest in some soils but not others. Furthermore, the effects of catechin on grasses in field experiments are highly variable among sites. There is also initial evidence that Centaurea is more allelopathic to North American native species than congeneric European native species in vitro and in the field, and these biogeographic differences have been suggested to be consistent with the “Novel Weapon Hypothesis” (NWH). This is the idea that some invaders may succeed because they possess unique allelopathic, defense or antimicrobial biochemistry to which naïve natives have not adapted.

Phytotoxicity has been repeatedly demonstrated, but the most controversial aspects of the potential role of catechin as an allelopathic compound are whether or not the roots exude enough catechin to be phytotoxic, and the conflicting reports on soil concentrations; are they high enough to be phytotoxic? Early reports of very high catechin concentrations in soils are not reliable due to sample contamination during analysis and the inability to consistently find such high concentrations in later field studies (Callaway RM and Vivanco JM, unpublished data). Blair et al. reported finding catechin in many soil samples but at low levels, never more than 1 μg g⁻¹, and argued that this concentration could not be phytotoxic. Perry et al., using a minimum detection limit of 25 μg g⁻¹, detected catechin in only 20 soil samples out of 402, but this was for a set of plants repeatedly measured over a season, and at one point in time all plants at the site were associated with catechin in the soil at a very high mean concentration (650 ± 450 (SD) μg g⁻¹). Pulses released of roughly similar concentrations have also occurred in mesocosms with C. maculosa (Schultze M and Paschke M, unpublished data). Extreme variation in detection may be due to pulsed releases, but catechin is highly ephemeral in soil, and applied concentrations of (±)-catechin result in far lower concentrations in solution, sand and soil than calculated from the application rate. This may be due to the interaction of the compound with components in the soils and oxidation. However, catechin detection and phytotoxicity may also be affected by soil microbial communities, a component of the system that has been minimally explored.

Catechin Phytotoxicity and Soil Microbial Communities

In this Addendum, we report the impact of catechin on soil microbial activity by measuring soil CO₂ release, and modification of catechin phytotoxicity in soil free from soil microbes. Levels of nitrogen (N) and phosphorus (P) in soil are influenced by microbial activity and death; we measured levels of these nutrients in catechin-treated soil.

Fifty g soil was placed in each of 9-cm Petri dishes, and treated with appropriate amount of catechin to get final concentrations of 133, 266 or 400 μg catechin/g soil (see for methodology). Based on
previous work\(^9\) (Fig. 2) these added concentrations to this particular soil probably created detectable or effective concentrations of >1, 1–5 and 1–40 \(\mu \text{g catechin}/\text{g soil}\). It is important to note that bulk soil concentrations of an allelochemical far overestimate the phyto-toxic dose because interactions can occur at root-root interfaces; however, these concentrations provide a reasonable place to start. Soil treated with the same amount of water served as controls. Ten seeds of \textit{Phalaris minor} or \textit{Brassica campestris} were placed on control or treated soil. Data on root and shoot length were collected after 7 days. Each experiment was replicated six times. Since CO\(_2\) release is a good indicator of microbial activity in different soils,\(^25\) we measured soil CO\(_2\) respiration by chemical titration following Andersen.\(^26\) Soil was treated with catechin to achieve added concentrations of 0, 133, 266 or 400 \(\mu \text{g catechin}/\text{g soil}\).\(^9\) Ten mL 0.1 N NaOH was placed in each 5-cm Petri dish, which was then placed in a chamber (433 cm\(^3\)) filled with 150 g control or treated soil. Chambers were then immediately covered and care was taken to avoid any loss of CO\(_2\). Soil was incubated for 24 h, and experiment was terminated by adding 1 mL of 0.1 N BaCl\(_2\) to NaOH. Ten mL of NaOH taken from blanks, controls and treatments was titrated against 0.1 N HCl, and the amount of CO\(_2\) released was calculated. Control and treated soils were analyzed for extractable phosphate-P using molybdenum blue method.\(^27\) To determine total organic N, soil was digested using the semi-micro Kjeldahl method, and N concentration was determined using the indophenol blue method.\(^27\)

When soil was sterilized, several concentrations of catechin increased shoot length (Fig. 1; \(F_{\text{Phalaris}} = 12.743, \text{df} = 3, 199, p < 0.001; F_{\text{Brassica}} = 7.316, \text{df} = 3, 229, p < 0.001\)) and root length (\(F_{\text{Phalaris}} = 10.722, \text{df} = 3, 199, p < 0.001; F_{\text{Brassica}} = 8.992, \text{df} = 3, 229, p < 0.001\)) and no concentration inhibited the growth of these species. In our previous study, catechin addition to this same soil, but not sterilized, inhibited plant growth at very low detectable concentrations (1.4 ± 1.4 to 36.1 ± 10.2 [1 SE] \(\mu \text{g g}^{-1}\)), which suggests that in some soils and for some species catechin may need to interact with soil microbial communities to cause plant growth inhibition. \textit{Phalaris} was inhibited in the initial experiment,\(^9\) and to our knowledge the only species in the Brassicaceae tested with catechin is \textit{Arabidopsis thaliana}, which is highly sensitive.\(^14\) We emphasize however, that in other experiments catechin has been shown to be phytotoxic in vitro and, river sand, and sterile sand,\(^9,16\) and thus these microbial effects are likely to be just one more component of the conditional effects of this species.
We observed a concentration-dependent decline in CO₂ efflux in non-sterile soil treated with catechin (F = 84.254, df = 3, 20, p < 0.0001) (Fig. 2A) suggesting that catechin killed microbes. (+)-Catechin has inhibitory effects on soil microbial density in vitro and this effect is far stronger on microbes that are found in North American soils than those from European soils.⁵ No significant differences were observed in the soil concentrations of PO₄-P (F = 1.591, df = 3, 20, p = 0.223). We observed higher total N levels in soil treated with 400 μg/g soil compared to untreated soil (t = -3.799, Pₕ-2-tailed = 0.004) (Fig. 2B inset), but there is no clear explanation for why suppressing total microbial activity would increase total soil N, unless all microbes but free living N₂-fixers were suppressed and N₂-fixers were enhanced. Acinetobacter calcoaceticus, which does not fix N₂, can utilize other forms of catechin as its sole carbon source²³ and genes involved in the utilization of various forms of catechin have been reported from the genomic DNA of Rhizobium sp. and Bradyrhizobium japonicum USDA 110, both N₂-fixers.²⁸ ²⁹

Our results indicate that catechin can inhibit general soil microbial activity and that in some soils the role of soil microbial communities may function as either drivers or passengers for catechin phytotoxicity. Either way, these results are a step towards a better understanding of the conditional effects of catechin.

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