Catechin secretion and phytotoxicity

Fact not fiction

Harsh P. Bais1,2 and Shail Kaushik1,2
1Department of Plant and Soil Sciences; University of Delaware; Newark, DE USA; 2Delaware Biotechnology Institute; Innovation Way; Newark, DE USA

Research indicates that the invasiveness of Centaurea stoebe is attributed to the stronger allelopathic effects on the native North American species than on the related European species, which is one of the unquestionable aspects of the “novel weapons hypothesis (NWH).” Studies originating from controlled to field conditions have shown that C. stoebe utilizes its biochemical potential to exert its invasiveness. The roots of C. stoebe secrete a potent phytotoxin, catechin, which has a detrimental effect on the surrounding plant species. Although studies on catechin secretion and phytotoxicity represent one of the most well studied systems describing negative plant-plant interactions, it has also sparked controversies lately due to its phytotoxicity dosages and secretion effluxes. Previous reports negate the phytotoxic and pro-oxidant nature of catechin.1-3 In our recent study we have shown that catechin is highly phytotoxic against Arabidopsis thaliana and Festuca idahoensis. We also show that (+) catechin applied to roots of A. thaliana induces reactive oxygen species (ROS) confirming the pro-oxidant nature of catechin. In addition, activation of signature cell death genes such as acd2 and cad1 post catechin treatment in A. thaliana ascertains the phytotoxic nature of catechin.

The secretion of catechin from the roots of the noxious weed, Centaurea stoebe is one of the best described examples of negative plant-plant interactions mediated through phytotoxins, which is also known as allelopathy.4 In the last eight years, various lines of research have shown that different invasive plants use a biochemical machinery to target recipient plant communities to invade in direct and indirect plant-plant interactions.4 It has been demonstrated that when applied to the roots of Arabidopsis thaliana, the phytotoxin (+) catechin triggers a wave of reactive oxygen species (ROS), leading to a cascade of genome-wide changes in gene expression and, ultimately, death of the root system.5 Biochemical links describing the root secreted phytotoxin, (+) catechin, represent one of the most well studied systems to describe negative plant-plant interactions. However, the original work on catechin has also sparked some controversies on phytotoxicity and pro-oxidant behavior of the secreted chemical. Earlier studies2-3 showed that catechin is not phytotoxic but it bears strong anti-oxidant activity. To add to this inconsistency, one of the original reports of catechin secretion has been recently retracted from the literature.6 The retracted note conveys that the authors of the original report failed to reproduce catechin secretion and phytotoxicity against Arabidopsis thaliana. In contrast, several other lines of work,7-23 some originating from the same research group showed catechin secretion to an order of mg g⁻¹ samples.5,7,14,15,18,20 It is valid to argue that the irreproducibility in catechin secretion observed by Stermitz et al. (2009)6 could be related to the factors such as instability of catechin in medium and sampling time. Since, Stermitz et al. (2009)6 didn’t dwell on the methodological difficulties that were encountered negating the detection of catechin in their repetition trials; it is hard to explain how
the same group.\textsuperscript{5,7,14,15,18,20} reported catechin in the secretions of \textit{C. stoebe} to a level of mg g\textsuperscript{-1} sample. In contrast, our recent data\textsuperscript{24} clearly shows that catechin is indeed phytoxic against \textit{A. thaliana} and \textit{Festuca idahoensis}. Duke et al. 2009 were unable to replicate the phytoxicity studies from previous reports\textsuperscript{5} mainly due to the fact that they did not follow the exact procedure as described by Bais et al. 2003. They used 3 seedlings per treatment versus one seedling per treatment. In addition, they used acetone as a solvent instead of methanol\textsuperscript{6} and used half strength MS medium in place of full strength. These differences, though seemingly trivial were enough to cause discrepancies in the data as elaborated in our results.\textsuperscript{24} Our data showed a clear enantiomeric dependent affect of (+) catechin, wherein (-) catechin isomer revealed a severe rhizotoxic response at 20 \textmu g ml\textsuperscript{-1} level.\textsuperscript{24} Root mortality patterns were also checked by time lapse movies, wherein seedlings treated with (+) catechin (100 \textmu g ml\textsuperscript{-1})/(-) catechin (10 \textmu g ml\textsuperscript{-1}) and (+) catechin (200–250 \textmu g ml\textsuperscript{-1}) were transferred on day 3 to MS plates without any catechins. Our recent time lapse movies show that seedlings treated with (-) catechin show strong mortality (as documented by no or reduced root growth) compared to (+) catechin isomer treated roots.\textsuperscript{24} In addition, the phytoxic effect was manifested equally in aqueous as well as organic phase against both \textit{A. thaliana} and \textit{F. idahoensis}. Interestingly, our data also revealed the presence of catechin in the growth medium of \textit{C. stoebe} to support a recent study.\textsuperscript{21} The authors reported high levels of catechin secretion from \textit{C. stoebe} hydroponic cultures compared to previous reports.\textsuperscript{3} Interestingly, these authors also reported that catechin secretion in \textit{C. stoebe} is diurnally regulated; wherein catechin degrades to catechol. Our results were concurrent with these findings indicating that the time of sampling could be an issue in detection of catechin. Furthermore, the unstable nature of the compound also adds to the variations in its detection and quantification.

Our original report that the phytoxicity of catechin treated roots is prompted by elevated levels of ROS was corroborated by the fact that catechin treatment results in the production of elevated levels of ROS.\textsuperscript{25-30} The argument that catechins bear a strong anti-oxidant activity\textsuperscript{3} is not very exciting, as catechins are reported anti-oxidants (SciFinder search as October 2008). However, the inference drawn by Duke et al. (2009) that catechins cannot bear pro-oxidant activity because they are anti-oxidants might be a little premature especially since the anti- and pro-oxidant capacity is correlated to the number of hydroxyl groups.\textsuperscript{25-28} Likewise, Sofic et al. (2001) have reported that anti to pro-oxidant shifts of catechins might be attributed to their hydroxyl and amine groups.\textsuperscript{28}

Furthermore, we showed that catechin phytoxicity is mediated through transcriptional upregulation of signature cell death genes such as accelerated cell death (\textit{acd2}) and constitutively activated cell death 1 (\textit{cad1}). We also reconfirmed the earlier observation that catechin induces reactive oxygen mediated (ROS) mediated phytoxicity in \textit{A. thaliana} and that catechin induced ROS is aggregated in presence of divergent transition metals. Catechin is known to bind to transition elements to enhance its phytoxicity and conditional allelopathic response.\textsuperscript{29,30} This supports our observation that ROS due to catechin-metal complex is responsible for modification of downstream signaling proteins contributing to altered gene expression and cell death.

While our results advocate the phytoxic and pro-oxidant nature of catechin, they also highlight the fact that this allelochemical may be perceived differently by plant communities when they are in monocultures versus isolated stands. The deviation in results reported previously\textsuperscript{24} could be due to different media conditions and a group effect in catechin treated seedlings. Our data proposes that precise conditions are needed to evaluate the overall effect of catechin secretion and toxicity.

References

1. Duke SO, Blair AC, Dayan FE, Johnson RD, Meepagala KM, Cook D, et al. Is (+) catechin a “novel weapon” of spotted knapweed (\textit{Centaurea stoebe})? J Chem Ecol 2009; 35:141-53.
2. Blair AC, Hanson BD, Brunk GR, Marris RA, Westra P, Nissen SJ, et al. New techniques and findings in the study of a candidate allelochemical implicated in invasion success. Ecol Lett 2005; 8:1039-47.
3. Blair AC, Nissen SJ, Brunk GR, Huffbauer RA. A lack of evidence for an ecological role of the putative allelochemical (+) catechin in spotted knapweed invasion success. J Chem Ecol 2006; 32:2357-51.
4. Weidenhamer JD, Callaway RM. Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. J Chem Ecol 2010; 36:56-69.
5. Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM. Allelopathy and exotic plant invasion: From molecules and genes to species interactions. Science 2003; 301:377-80.
6. Stermitz FR, Huffbauer RA, Vivanco JM. Enantioselective-dependent phytotoxicity and antimicrobial activity of (+) catechin. A rhizosorbed racemic mixture from spotted knapweed. Plant Physiol 2009; 151:967-76.
7. Weir TL, Bais HP, Stull VJ, Callaway RM, Thelen GC, Rideroun WM, et al. Oxalate contributes to the resistance of \textit{Gaillardia grandiflora} and \textit{Lupinus sericeus} to a phytotoxic produced by \textit{Centaurea maculosa}. Planta 2006; 223:785-95.
8. He WM, Feng Y, Rideroun WM, Thelen GC, Pollock JL, Diaconu A, et al. Novel weapons and invasion: Biogeographic differences in the competitive effects of \textit{Centaurea maculosa} and its root exudate (+) catechin. Oecologia 2009; 159:803-15.
9. Inderjit, Pollock JL, Callaway RM, Holben W. Phytoxic effects of (+) catechin in vitro, in soil, and in the field. PLoS ONE 2008; 3:2536.
10. Rideroun WM, Vivanco JM, Feng YL, Horiiuch J, Callaway RM. No evidence for trade-offs. \textit{Centaurea} plants from America plant better competitors and defenders. Ecol Mono 2008; 78:369-86.
11. Thorpe AS, Thelen GC, Diaconu A, Callaway RM. Root exudate is allelopathic in invaded community but not in native community: Field evidence for the novel weasels hypothesis. J Ecol 2009; 97:641-5.
12. Prithiviraj B, Perry LG, Bader DW, Vivanco JM. Chemical facilitation and induced pathogen resistance mediated by a root-secreted phytox. New Phyotol 2007; 173:852-60.
13. Veluri R, Weir TL, Bais HP, Stermitz FR, Vivanco FM. Phytoxic and antimicrobial activities of catechin derivatives. [Apric Food Chem 2004; 52:1077-82.
14. Perry LG, Thelen GC, Rideroun WM, Weir TL, Callaway RM, Paschke MW, et al. Dual role for an allelochemical: (+) catechin from \textit{Centaurea maculosa} root exudates regulates conspecific seedling establishment. J Ecol 2005; 93:1126-35.
15. Thelen GC, Vivanco JM, Newingham B, Good W, Bais HP, Landres P, et al. Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. Ecol Lett 2009; 12:216-27.
16. Weir TL, Perry LG, Vivanco JM. Phytoxins produced by invasive weeds and their applications in agriculture and the restoration of natural areas. In Natural Products for Pest Management (Rimando AM, Duke SO, eds.). Symposium Series No. 92. Washington D.C.: American Chemical Society 2006; 99-112.
17. Perry LG, Weir TL, Prithiviraj B, Paschke MW, Vivanco JM. Root exudation and rhizosphere biology: multiple functions of a plant secondary metabolite. In Communication in Plants (Manuska FBS, Volkman D., eds.) Berlin: Heidelberg: Springer-Verlag 2006; 403-20.
18. Perry LC, Thelen GC, Rideroun WM, Callaway RM, Paschke MW, Vivanco JM. Concentrations of the allelochemical (+) catechin in \textit{Centaurea maculosa} soils. J Chem Ecol 2007; 33:237-45.
19. Simes K, Du J, Korreschmas FS, Broeckling CD, Stermitz FS, Vivanco JM, et al. Phytotoxic catechin leached by seeds of the tropical weed \textit{Sesbania virgata}. J Chem Ecol 2008; 34:681-7.
20. Broeckling CD, Vivanco JM. A selective, sensitive and rapid in-field assay for soil catechin, an allelochemical of \textit{Centaurea maculosa}. Soil Biol Biochem 2008; 40:1189-96.
21. Tharayil N, Triebwasser DJ. Elucidation of a diurnal pattern of catechin exudation by \textit{Centaurea stoebe}. J Chem Ecol 2010; DOI: 10.1007/s10886-100-9749-7.
22. He WM, Feng Y, Ridenour WM, Thelen GC, Pollock JL, Diacona A, et al. Novel weapons and invasion: biogeographic differences in the competitive effects of Centaurea maculosa and its root exudate (α) catechin. Oecologia 2009; 159:803-15.

23. Pollock JL, Callaway RM, Thelen GC, Holben WE. Catechin-metal interactions as a mechanism for conditional allelopathy by the invasive plant, Centaurea maculosa. J Ecol 2009; 6:1234-42.

24. Kaku M, Nakagawa N. (α) catechin with Cu²⁺ induces protein modifications via reactive oxygen species-independent pathway. J Health Sci 2009; 3:441-6.

25. Okawa S, Furukawa A, Asada H, Hirakawa K, Kawanishi S. Catechins induce oxidative damage to cellular and isolated DNA through the generation of reactive oxygen species. Free Radic Res 2003; 37:881-90.

26. Sofic E, Denisova N, Youdim K, Vatenjak-Velagic V, De Filippo C, Meumedagic A, et al. Antioxidant and pro-oxidant capacity of catecholamines and related compounds: Effects of hydrogen peroxide on glutathione and sphingomyelinase activity in pheochromocytoma PC12 cells; potential relevance to age-related diseases. J Neural Transm 2001; 108:541-57.

27. Kaushik S, Venkatachalam L, Biedrzycki M, Bais HP. Catechin is a phytotoxin and pro-oxidant secreted from the roots of Centaurea stoebe. Plant Signal Behav 2010; 9:1-11.

28. Nanto F, Goto K, Seto R, et al. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. Free Radic Biol Med 1996; 21:895-902.

29. Foreman J, Demidchik V, Bothwell JHF, et al. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 2003; 422:442-6.

30. Cao J, Xu Y, Chen J, et al. Chemopreventive effects of green and black tea on pulmonary and hepatic carcinogenesis. Toxicol Sci 1996; 29:244-50.