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# Plants, prions and possibilities: Current understanding and significance of prion uptake into plants

Tracy A. Nichols

*U.S. Department of Agriculture*, [tracy.a.nichols@aphis.usda.gov](mailto:tracy.a.nichols@aphis.usda.gov)

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# PLANTS, PRIONS AND POSSIBILITIES:

## Current Understanding and Significance of Prion Uptake into Plants

By Tracy A. Nichols, Ph.D., M.S., M.A., Molecular Biologist  
Chronic Wasting Disease  
National Wildlife Research Center, USDA/APHIS/Wildlife Services  
Fort Collins, Colorado

**C**hronic wasting disease (CWD) is an infectious, neurodegenerative disease of deer (white-tailed and mule), elk, moose, sika deer and muntjac caused by a misfolded version of a normally occurring protein. The notion that CWD could be spread indirectly via the environment has been documented and accepted in the scientific community for quite some time. Deer and elk consume soil, inhale dust and lick objects that have infectious material on them, resulting in chronic, low dose exposure. Surface contamination of plants with urine or feces is likely an additional source

of exposure via ingestion and has been modeled in the laboratory by Pritzkow, et. al., (2015). In 2014 Dr. Christopher Johnson and his colleagues at the United States Geological Survey (USGS) and the University

of Wisconsin, Madison proposed a novel mechanism for CWD infectivity associated with plants at the international Prion meeting. Dr. Johnson and his group hypothesized and presented data supporting the idea that plants could take up infectious prion proteins into their tissues by contact and absorption through their roots. Although the idea of a plant pulling a protein into its roots seems a bit science fiction, it is in fact all science and no fiction. As early as the 1930s scientists documented the uptake of viruses into plant stems and leaves from the roots. Pathogen internalization by plants and its implications for human health has been widely studied. In a review article by Hirneisen et. al., (2013) the authors list 19 studies that showed bacteria, such as *E. coli* and salmonella, could infiltrate plants from the roots, and four studies demonstrating infiltration with such viruses as polio, canine calicivirus, hepatitis A and murine norovirus. In 1960 Jensen, McLaren and Jacobson demonstrated the ability of plants to pull proteins from the soil into their roots and were able to track their progress into roots using radioactively labeled proteins. More recent work by Paungfoo et. al., (2008) utilized fluorescent proteins to demonstrate that plants can pull proteins out of the soil and utilize them as a source of nitrogen if needed. It is therefore reasonable to question whether the prion protein could be taken up when it is an entire order of magnitude smaller (~10 nm) in diameter than bacteria such as *E. coli* (~2µm).

The USGS group is in the process of publishing their findings, but presented their data at the international Prion meeting in 2015 which demonstrated uptake into the roots of *Arabidopsis* (a common research plant), alfalfa, barley

and tomato plants by utilizing confocal microscopy to see fluorescently tagged infectious prion proteins. They were also able to detect the infectious prion using an amplification assay called protein misfolding cyclic amplification (PMCA) in the leaves of *Arabidopsis*, barley, alfalfa and maize. The stems and leaves of *Arabidopsis* were injected into the brains of a mouse model and were able to cause disease.

Two groups have published on the subject in the scientific literature, thus far. The first, Rasmussen et. al., (2014) failed to show prion uptake into wheat. However, this study utilized

detection assays (Western blot, and Idexx and Bio-Rad ELISA) that are not sufficiently sensitive to detect low levels of infectious prions. The other paper by Pritzkow, et. al., (2015) utilized the amplification assay PMCA mentioned above to look

for infectious prions in the stems and leaves of barley plants grown in contaminated soil. Care was taken to prevent surface contamination from the soil. The authors were able to detect low levels of infectious prion protein in the stems of the barley after a 3-week period of growth on contaminated soil. They also estimated that the stems had approximately the same level of infectivity as that of a 10<sup>-6</sup> to 10<sup>-9</sup> dilution of infectious brain homogenate.

A research group under the supervision of Dr. Mark Zabel at the Colorado State University Prion Research Center is in the process of publishing their findings on plants as well. This group examined four types of native plants (multi-leaf and grass) from a heavily contaminated CWD-endemic area. They are currently assessing prion contamination in these naturally exposed samples by PMCA, and rodent model tests to determine if transmission from these naturally exposed plants is possible.

It seems clear that prions can be on and inside of plants of various types, however it is currently unclear if the levels present in natural settings are sufficient to cause disease. The answer to this question is of considerable importance for both animal and human health. Currently at the USDA National Wildlife Research Center we are collaborating with the group from the USGS to answer this question utilizing an herbivorous rodent model and plant material procured from a heavily contaminated agricultural area. Because low dose animal studies are time consuming by nature, it will be a few years before the results are complete to answer the question.

**"It seems clear that prions can be on and inside of plants of various types, however it is currently unclear if the levels present in natural settings are sufficient to cause disease."**