

Accepted Manuscript

Endophytes: An Emerging Tool for Biological Control

Paul A. Backman, Richard A. Sikora

PII: S1049-9644(08)00083-2

DOI: [10.1016/j.biocontrol.2008.03.009](https://doi.org/10.1016/j.biocontrol.2008.03.009)

Reference: YBCON 2045

To appear in: *Biological Control*

Received Date: 3 March 2008

Accepted Date: 15 March 2008



Please cite this article as: Backman, P.A., Sikora, R.A., Endophytes: An Emerging Tool for Biological Control, *Biological Control* (2008), doi: [10.1016/j.biocontrol.2008.03.009](https://doi.org/10.1016/j.biocontrol.2008.03.009)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction and Commentary to a Special Issue of Biological Control

Endophytes: An Emerging Tool for Biological Control

Paul A. Backman^a and Richard A. Sikora^b,

^a*The Pennsylvania State University, Dept. of Plant Pathology, University Park, PA 16802 USA*

^b*University of Bonn, INRES-Phytomedicine Soil-Ecosystem Phytopathology & Nematology
Nussallee 9, D-53115 Bonn, Germany*

Received:

Accepted:

Available Online:

Scope and background of this compilation

In 2003, the Biological Control Committee of the American Phytopathological Society (APS) suggested that the time was right to develop a symposium on endophytes for the annual meeting of the Society to be held in 2005. We were charged with developing a series of topics and speakers that would address the status of endophytes for biological control of plant diseases. That symposium was held in the 2005 meeting of APS, July 30-August 3 in Austin, Texas, where it generated very strong attendance. Preliminary abstracts of presentations were published for that meeting (Various, 2005). Authors from these presentations are largely represented in this compilation. In addition, we have added additional papers on fungal endophytes for plant disease and insect management.

The nature and ecology of endophytes

In 1991, the mycology committee of APS held a discussion session on the emerging issue of endophytic fungi in grasses and woody plants, which eventually led to the publication of a book on the ecology and evolution of these endophytes (Redlin and Carris, 1997). In this book Rodriguez (1997) concluded that there were fungi in all plants, and that endophytes are widespread in nature. The majority of the book dealt with clavicipitaceous fungi that mediated reduced herbivory in grasses (from insect and mammalian herbivores), but also reported extended latent periods for some pathogens particularly in soybean and woody plants that seemed to be very similar to endophytic relationships. Little mention was made of endophytic prokaryotes.

Soon thereafter, a second book was published that was based on a symposium of the International Symbiosis Society (Bacon and White, 2000) and expanded the context to include prokaryotes and mycorrhizae, as well as grass and woody plant endophytic fungi. Kobayashi and Palumbo (2000) provided an extensive literature review and list of culturable bacterial endophytes that had been reported. Further, during this period, reports were made by several researchers using endophytes as carriers of novel genes developed by molecular tools. Particularly, Li et al. (2007) augmented, *Clavibacter xyli* subsp. *cynodontis* (*Cxc*) (syn. *Leifsonia xyli* subsp. *cynodontis*) with DNA encoding insecticidal proteins from *Bacillus thuringiensis* subsp. *kurstaki*. Another report inserted a β -1,3 glucanase gene into *Cxc* as a defense gene against fungal pathogens (Haapalainen et al., 1998). *Cxc* is a xylem-limited pathogen of another host (causal agent of sugar cane ratoon stunt disease), but is symptomatically latent in corn or rice xylem, stimulating the production of plant defense products. There are however small reductions in yield when compared to plants without endophytes when both are grown in the absence of pests.

The term endophyte, with the exception of the endotrophic mycorrhizal fungi, was always closely associated with beneficial organisms colonizing the phyllosphere. However, the definition of an endophyte is now broadened by many researchers and can include any organisms that live in plant tissue whether neutral, beneficial or detrimental (Sikora et al., 2007). A number of the papers presented in this volume demonstrate the impact that endorhizal microbial biodiversity has on plant health.

The diversity of the phyllosphere

The phyllosphere community is composed of epiphytes on the surface of aboveground plant parts, and endophytes in the internal tissues. The endophyte as we know it is a microbe that lives within the plant that is neutral or beneficial to the plant that hosts it. Typically, these are bacterial or fungal, and may be of three types: 1) pathogens of another host that are nonpathogenic in their endophytic relationship; 2) nonpathogenic microbes; and 3) pathogens that have been rendered nonpathogenic but still capable of colonization by selection methods or genetic alteration. Recent papers indicate that the

phyllosphere is much more diverse than previously thought. Using molecular techniques, studies by Yang et al. (2001) evaluating cultivated citrus, and Lambais et al. (2006) evaluating nine tree species in a subtropical forest, both determined a high level of diversity with many unculturable and often unidentifiable species. Further, Lambais estimated that by applying the diversity he found in his nine tree species to the estimated 20,000 vascular plant species in the forest, there would be between 2 and 13 million new microbial species identified in that forest alone. How this diversity relates to the ecological functions of these organisms and how these functions are related to the metabolic capabilities of microbe and plant are just now beginning to be studied. More pertinent to this discussion, the roles of these often unique microorganisms in protecting the plant against insect and pathogen attacks are little understood but compelling in their possibilities.

The diversity of the endorhiza

Microbial communities in the endorhiza, both bacterial and fungal, have been shown to be important regulators of root health, whether they are obligate symbionts or saprophytic mutualists. Their presence has been shown to have a greater impact on plant health than those active in the rhizosphere. The fact that they are saprophytic, colonize multiple plant species, can control both pests and diseases, and have unique mechanisms of action, make them both scientifically and commercially important.

There are astonishing numbers of microorganisms capable of colonizing the endorhiza, commensally, mutualistically, or pathogenically. Knowledge of the true size and function of the microbes making up these communities is scarce. Some of the more beneficial interactions are presented in this issue. The number of fungal species colonizing the endorhiza of banana, for example, exceeded 130 with an additional 75 detected in the center of corm tissue (Sikora et al., 2003). High numbers of fungal genera grow endophytically in the roots of all crops and have for the most part been overlooked in favor of research on pathogens.

Population densities of bacterial endophytes have been shown to be greatest in plant roots (McInroy and Kloepper, 1995) with densities ranging from 10^4 to 10^6 CFU per g fresh weight in cotton and sweet corn roots. In potato, the average bacterial densities on a CFU per g fresh weight basis over two seasons were: 5.6×10^7 in the rhizosphere, 2.2×10^6 in the endorhiza, and 5.2×10^5 phyllosphere and were lowest in the endosphere 3.9×10^4 (Berg et al., 2005). The total number present at anyone time controlled by the plant and environment (Hallmann et al., 1997)

Implications of host defenses facilitated by endophytes

This special issue of Biological Control provides a series of articles that indicate the benefits of selected fungal or bacterial endophytes in protecting the plant against attack by pests. Unlike the natural condition where the plant may be expressing the benefits of a consortium of protective organisms, all of these articles point to one organism and the associated health benefits that it provides to a plant. The use of multiple organisms in a consortium that will coexist in the internal plant tissues is just beginning to be undertaken. The results presented on the identification of *in planta* suppressiveness to nematodes in banana induced by a community of antagonistic fungal endophytes, demonstrates that microbial consortia effectively impact plant health in nature. Additionally, if the components of a consortium independently triggers plant defense cascades as pathogens of other hosts (SAR), nonpathogens (ISR), as well as certain fungi to trigger insect resistance (Jasmonate pathway), consortia may provide the answer to providing higher levels of durable pest resistance in plants. Melnick (Melnick, initials?, give affiliation, 2008 unpublished; I take it that you have the author's permission to refer to these unpublished data; if not please get approval) found that endophytic bacteria could persist at least 4 months in cacao leaves that were directly treated. This durable resistance is made even more likely if added microbes produce defense proteins or antibiotics that function independent of the defense cascades just mentioned. Obviously, plants growing in the field will contain microbial consortia. We as scientists will be challenged to find memberships that are compatible, deliverable, and effective. There is an additional challenge: If the crop is a perennial, or is an established annual, our added

organisms will likely have to compete with existing microflora that are already in the tissues at the time of application.

In the papers presented here one mechanism stands out -- the induction of systemic resistance in plants following treatment with endophytic fungi or bacteria. The mechanisms of action extend beyond our present knowledge and opens new lines of research on the biochemical and genetic nature of signaling and gene induction.

The endophytic interactions presented in this series of papers opens up new avenues for research on functional genomics. Genomic analysis could lead to discovery of new genes important in regulating pathogen survival and infection processes. Systemic changes in root exudates that lead to the reduced attractiveness to nematodes or suppression of spore germination need deeper examination. An understanding of the interrelationships between endophytes and the plant genome could lead to the identification of genes for resistance that are up-regulated only in the presence of a beneficial microbe. These genes may promote the development of innovative plant protection compounds based on unique mechanisms.

Technological impact

Unique endophytes could be used directly to treat seeds or transplants limiting substantially the side-effects of abiotic and biotic factors on the biological agent by almost immediately protecting them within plant tissue. In the endosphere, mutualistic endophytes are in protected environments that give them a competitive advantage over organisms of the rhizosphere and phyllosphere -- consistent nutrient flow, pH, moisture, as well as protection from high numbers and densities of competitors.

Also important is the fact that the organisms occupying the endosphere are not accidentally there but most probably have been selected for this niche by the plant, because of the beneficial effects they offer their host, and their abilities to resist the effects of plant defense products. The energy lost by the plant in the production of

endophyte biomass is in all likelihood adequately compensated for by the improvements in plant health derived from the presence of mutualistic microorganisms.

In the end, the amount of inoculum required to protect the host on a per hectare basis is minimized with the precise targeting of endophyte treatments. This should make the approach attractive to the biotechnology industry looking for alternatives to traditional pesticides, as targeting the pathosphere of pathogen, insect and nematode infections assures improved efficacy.

The future use of biological-chemical combinations of endophytes in combination with commercial pesticides applied to the seed or seedling could lead to synergistic effects on one or multiple disease causing agents. The chemicals could provide near instantaneous suppression on pathogenic organisms, while the biological agent could provide continuing control well into the crops production cycle. IPM on the seed reduces costs and environmental impact, while allowing the biological agent to build up momentum for biological control.

Acknowledgments

The authors wish to thank the American Phytopathological Society and its Biological Control Committee for sponsoring the symposium that led to the development of this collection of papers. We also thank XXXXXXXXXX for the photographs that are part of the cover page of this special issue of Biological Control.

References

Bacon C.W. White, J.F. Jr. (Eds.) 2000. Microbial Endophytes. Marcel Dekker, N.Y. 487p.

Berg, G., Krechel, A., Ditz, M., Sikora, R.A., Ulrich, A., Hallmann, J. 2005. Endophytic and ectophytic potato-associated bacterial communities differ in structure and

antagonistic function against plant pathogenic fungi. *FEMS Microbiology Ecology* 51, 215-229.

Haapalainen, M. L., Kobets, N., Piruzian, E., Metzler, M.C. 1998. Integrative vector for stable transformation and expression of a β -1,3-glucanase gene in *Clavibacter xyli* subsp. *Cynodontis*. *FEMS Microbiology Letters* 162, 1-7.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F., Kloepper, J.W. 1997. Bacterial endophytes in agricultural crops. *Canadian J. Microbiology* 43, 895-914.

Kobayashi, D.Y. Columbo, J.D. 2001. Bacterial endophytes and their effects on plants and uses in agriculture. In: *Microbial endophytes*, Bacon C.W. White, J.F. Jr. Marcel Dekker, NY, pp 199-236. .

McInroy J.A., Kloepper, J.W., 1995. Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant and Soil* 173, 337-342.

Redlin, S.C. Carris, L.M. (Eds.) 1997. *Endophytic Fungi in Grasses and Woody Plants: Systematics, Ecology and Evolution*. APS Press, St. Paul, MN, 223p.

Rodrigues, K.F. 1997. Fungal Endophytes of Palms. In: *Endophytic fungi in grasses and woody plants: systematics, ecology and evolution*. APS Press, St. Paul, MN, pp 121-132. 223p.

Lambais, M.R., Crowley, D.E., Cury, J.C., Büll, R.C., Rodrigues, R.R. 2006. Bacterial diversity in tree canopies of the Atlantic forest. *Science* 312,1917.

Li, T.-H., Zeng, H.-., Ping, Y., Lin, H., Fan, X.-L. Guo, Z.-G., Zhang, Z.-G. 2007. Construction of a stable expression vector for *Leifsonia xyli* subsp. *cynodontis* and its application in studying the effect of the bacterium as an endophytic bacterium in rice. *FEMS Microbiology Letters* 267, 176–183

Sikora, R.A., Niere, B., Kimenju, J. 2003. Endophytic microbial biodiversity and plant nematode management in African agriculture. In: Biological Control in IPM Systems in Africa. Eds. P. Neuenschwander, C. Borgemeister, U. Langewald. CABI Publishing UK, pp 179-192.

Sikora, R.A., Schäfer, K., Dababat, A.A. 2007. Modes of action associated with microbially induced *in planta* suppression of plant-parasitic nematodes. Australasian Plant Pathology 36,124-134.

Yang, C-H., Crowley, D.E. Borneman, J, Keen, N.T. 2001. Microbial phyllosphere populations are more complex than previously realized. Proc. Natl. Acad. Sci., US 98, 3889-3894.

ACCEPTED MANUSCRIPT