Tolerance of *Medicago* Legumes to Biotic Stresses: Integrating Genomic Function with Plant Response to Pests

A proposal for a multidisciplinary conference involving
cellular biology and genomics, plant physiology and biochemistry, and crop protection

Summary

Medicago represents a genus of plants that is of great value in forage production and environmental restoration. Yet, these plants are subject to a wide range of pests, most notably from herbivorous insects and pathogenic fungi. Currently, several sets of researchers (NA and European) are describing and investigating the functional genomics of *Medicago* species. Since plant genotypes vary in their ability to tolerate pests, genomic function is related to the protection of *Medicago* species from pest-induced injury. As specialists in crop protection and physiology of *Medicago*, we propose to chair a multidisciplinary conference to identify linkages between genomic function, plant physiology and biochemistry, and plant-pest interactions, leading to increased awareness and greater collaboration among disciplines. The outcome of the conference will be a state-of-the-art review of tolerance of *Medicago* legumes to biotic stresses, as well as a list of specific research needs. The ultimate goal is to understand mechanisms of tolerance to pests within *Medicago*, and to produce new, improved varieties and germplasms that withstand pest attack.

Project Description

(1) Justification

Alfalfa, *Medicago sativa*, is the fourth most widely cultivated agronomic crop in the United States and the principal forage component of a wide variety of agricultural production and conservation systems throughout the U.S. Between 10 and 11 million hectares of alfalfa are grown throughout all 50 states. Although having a cash value of $8 billion in 2002, the true worth of alfalfa and other forages to these systems is generally underestimated because their value is marketed indirectly as meat ($70 billion), wool ($660 million), or dairy products ($25 billion). In addition, alfalfa contributes to the sustainability of rotational crop production systems because of its perennial nature and its ability to fix nitrogen.

Stresses imposed by factors such as unfavorable growing conditions, interference by weeds, and injury by pathogens, nematodes, and arthropod pests significantly reduce alfalfa yield, reduce forage quality, and shorten stand life (Table 1). New genetic tools, and specifically new research centered on *Medicago truncatula* genomics, provide opportunities for increasing the tolerance of alfalfa to these stresses. The goal of the proposed conference is to bring together key researchers of *Medicago* for the purpose of identifying key linkages between genome structure and function, plant physiology and biochemistry, and plant protection from insect and pathogen pests, leading to increased awareness and greater collaboration among disciplines, and ultimately to increased tolerance of alfalfa to biotic stresses.

Table 1. Pest complexes contributing directly to biotic stress on forage alfalfa, impacting production, quality, and stand persistence in the central and eastern United States as identified by research and extension personnel within Regional Research Committee NC-226.

Pest complex	Specific causal groups	Examples
Herbivorous insects	Root feeders Foliar and vascular feeders	*Sitona hispidulus, Otiorhynchus ligustici, Hypera postica, Acyrthosiphon kondoi, Empoasca fabae*
Root/crown diseases	Fungal pathogens Parasitic nematodes	*Phytophthora medicaginis, Fusarium oxysporum, Aphanomycetes eutiches, Ditylenchus dipsaci, Meloidogyne hapla*
Foliar/wilt diseases	Fungal pathogens Viral pathogens Bacterial pathogens	*Verticillium albo-atrum, Psuedopeziza medicaginis, Phoma medicaginis,* alfalfa mosaic virus, *Clavibacter michiganense*
Seedling diseases	Fungal pathogens Parasitic nematodes	*Sclerotinia trifoliorum, Pythium* spp., *Aphanomycetes eutiches, Phytophthora megasperma, Meloidogyne hapla*

Impact and Management of Pests. Diseases and insect pests are among the most significant factors limiting yield and quality of forages (Beuselinck et al. 1994, Hanson et al. 1988). Considerable research has focused on reduction of crop productivity because of damage by pests without considering the mechanisms involved in these reductions (Poston et al. 1983). The absence of broad explanations, or even hypotheses, regarding effects of specific types of insect injury on alfalfa physiology and biochemistry requires that mechanisms of injury are tested separately for every pest species. Research relating pest-induced injury and the physiological and biochemical response of the crop would represent the foundation for broader considerations of biotic stressors on alfalfa and for developing general hypotheses that predict specific impacts of other pests to various types of crop injury (Baldwin and Preston 1999). Delineating injury-induced changes in host physiology and biochemistry is fundamental to explaining interactions between stressors, and the lack of such an understanding has been an impediment to the development of realistic simulation models for interactions between alfalfa and biotic stressors (Pennypacker et al. 1990, Lamp et al. 2004). Ultimately, our ability to efficiently reduce losses in alfalfa from biological stressors will be constrained as long as we cannot characterize the physiological, and ultimately the biochemical and genetic, mechanisms behind these stresses (Peterson and Higley 2001).

Development of disease- and insect-resistant alfalfa varieties has been a major focus of public institutions and private seed companies. Resistance has improved to such diseases as bacterial wilt, Phytophthora root rot, and Anthracnose, and to such insects as potato leafhopper (e.g., Stuteville and Erwin 1990, Elden and McCaslin 1997). However, diseases and insect pests still threaten the alfalfa crop each year, and in some cases severe losses occur. For example, spring black stem caused by *Phoma medicaginis*, f. sp. *medicaginis*, lepto leaf spot, caused by *Leptosphaerulina briosiana*, summer black stem, caused by *Cercospora medicaginis*, rust, caused by *Uromyces striatus*, and Stemphylium leaf spot, caused by *Stemphylium* spp., may cause serious yield losses and reductions in forage quality as a result of physiological disruption in normal alfalfa physiology (Broscious et al. 1987, Nutter et al. 2002, Rhodes and Myers 1986, Sulc and Rhodes 1997). Among insects, potato leafhopper, *Empoasca fabae*, still commonly exceeds economic thresholds in spite of the release of new glandular-haired varieties targeted for its suppression (Hansen et al. 2002). Other key insect pests of alfalfa include alfalfa weevil, spotted alfalfa aphid, and alfalfa snout beetle, each of which influence alfalfa physiology (Sulc and Lamp *in press*). Although varieties exist that demonstrate some tolerance to each of these pests (e.g., Lefko et al. 2000), tolerance as a mechanism of host plant resistance has not been successfully deployed, in part because of a lack of understanding of mechanisms. For example, plants should be capable of recognizing the attack of a pest, and the plant's defense in response likely involves the expression of specific genes (Wang et al. 2004). An understanding of these mechanisms would clearly aid in the development of host plant resistance and tolerance.

Integrated pest management (IPM) is a system of pest control that seeks to manage pests using economically efficient and environmentally sound approaches (Cate and Hinckle 1994; Bottrell 1979). It relies primarily on preventive approaches, such as host plant resistance, biological control, and cultural practices to maintain pest populations and their injury below damaging levels. If preventive approaches fail, then responsive practices, such as the application of pesticides, are used on the basis of pest densities or disease incidence and the comparison to established economic thresholds. In recent years, new terms that are essentially the same as IPM have developed, e.g. biologically intensive integrated pest management (Frisbee and Smith 1989), biologically-based pest management (U.S. Congress 1995), ecologically-based pest management (National Research Council 1996), and total system approach to sustainable pest management (Lewis et al. 1997). The new terms place even more emphasis on sustainable approaches for pest control, with the goal "to restore and preserve balance to the managed ecosystem by duplicating natural processes to the maximum extent possible" (National Research Council 1996, p. 115). Tolerance to biotic stressors is one of the key methods for pest management in the future.

Tolerance (also called plant compensation) is defined here as the ability of a crop genotype to suffer little or no loss subsequent to pest-induced stress relative to the uninjured state, and is well known as one of the mechanisms of host plant resistance (Painter 1958). It differs from other forms of resistance in that tolerance does not have any selective impact on pest populations, providing for a more sustainable method for managing pests without pest adaptation. Research of mechanisms for tolerance has focused on resource allocation patterns, plant architecture, and specific physiological traits (Trumble et al. 1993, Stowe et al. 2000). We believe that for tolerance to be developed for managing pests in alfalfa, research is also needed on the biochemical and genetic basis for tolerance.

Recent Research on Alfalfa Biochemistry and Genomics. Decades of research on alfalfa have failed to reveal the mechanisms that lead to poor plant persistence and stand loss due to abiotic stresses. Time and again low levels of root reserves, primarily starch and sugars, have been implicated as the primary cause of premature death of alfalfa (Graber et al. 1928, Smith 1962). Recent results have revealed that this is a serious oversimplification, and that other physiological and biochemical mechanisms determine whether an alfalfa plant dies or survives (Avice et al. 1996, Volenec et al. 1996, Meuriot et al. 2003). There is a clear need for a new paradigm if we are to discover the key processes and their regulatory genes that directly impact alfalfa growth and persistence (e.g., Haagenson et al. 2003).

Genetic selection continues to be the primary mechanism by which we increase yield and stress tolerance of alfalfa (Volenec et al. 2002). However, as in many crop species, future improvements by genetic manipulation will depend on new insights into basic physiological and biochemical plant processes. The limiting factor in this area is that we lack knowledge of discrete traits or genes controlling stress tolerance and persistence of alfalfa that can serve as targets for manipulation. New genetic techniques, such as microarrays (Gibson 2002), may aid those discoveries. Our long-term goal is to improve yield and persistence of alfalfa by identifying and manipulating genes that affect the response of alfalfa to biotic stress. From the proposed conference will emerge a much better understanding of physiological processes and molecular events that relate biotic stresses to tolerance of alfalfa.

To discover more about the structure and function of genes in *Medicago*, *M. truncatula* was chosen for genome sequencing because of its relatively small genome, its short generation time, and high transformation efficiency (Cook 1999). Unlike alfalfa, which is tetraploid and an obligate outcrossing species, *M. truncatula* has a diploid genome (2 sets of 8 chromosomes) and is self-pollinated. Genes from *M. trunculata* possess high levels of sequence similarity to their counterparts from alfalfa, including genes that allow for the establishment of nitrogen fixing Rhizobia and pathogen resistance. Thus, the Samuel Roberts Noble Foundation established the Center for *Medicago* Genomics Research in 1999. The Center's goal is to develop a program that will make a significant impact in the areas of legume molecular biology, biochemistry, and genetics research (Noble 2004). The Noble Foundation has agreed to serve as host for this conference.

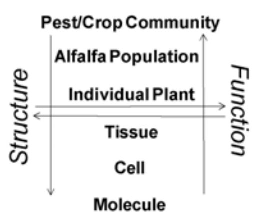
Development of the Conference. The NC-226 Multistate Project, "Development of Pest Management Strategies for Forage Alfalfa Persistence," provided the staging point for the development of the proposed conference. NC-226 recently ended its 5-year program, with a number of notable results (reports available at http://persist.umd.edu):

- Researchers in 3 states have discovered that alfalfa plant mortality primarily occurs in the summer, not in the winter. This is especially significant since extension specialists and breeding programs have focused on the abiotic stress of cold tolerance to overcome winter mortality. Summer mortality is

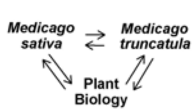
associated with biotic stress, including stresses imposed by insect herbivores and diseases.
- The mechanisms of biotic stresses include complex interactions between the biology of each pest species and the resulting interaction between alfalfa physiology and biochemistry.
- The dogma of carbohydrate cycling as a key feature in alfalfa persistence is now being expanded to include the importance of taproot protein reserves and cycling of nitrogen from root to regrowing shoots as key to growth and persistence of alfalfa.
- Testing of genotypes for susceptibility to physiological disruption by pests has indicated that tolerance may be a key mechanism for host plant resistance in alfalfa by the vascular-feeding potato leafhopper.

A recent discussion of the continuation of the NC-226 Regional Research project highlighted the need to expand research activities to include links from their current population, organismal, and physiological research with the genomic research using *M. trucatula* as a model organism. The conference was proposed as a critical need for planning future research needs.

We envision the conference as the vital means for information transfer and research-need identification because of the complexity of *Medicago* biology. In the diagram below, we have illustrated the flow of information across the hierarchy of life as vertical connection, and the flow between structure and function on any given level as horizontal. The vertical flow is especially critical because crop production and protection goals are determined at the upper three levels, yet many of the mechanisms used to explain field-level patterns of stress tolerance exist at the lower levels. Experiments that cross the hierarchical levels are critical for connecting mechanisms to field-level patterns. The utility of genetic-based tolerance has research needs across all levels. The horizontal connection is needed as well, linking the structure (e.g. population characteristics, plant morphology, biomass allocation) with function (e.g., plant survival rates, photosynthesis, respiration, translocation, nitrogen fixation). For the conference, this linkage is especially important because the mechanisms of tolerance likely work on function and have often evaded research centered on structure. Clear representation of the role of tolerance in structure and function of *Medicago* at each level is needed, as well as the definition of critical linkages across vertical levels.



The *Medicago* system is also complex because of the nature of the chromosome structure. Thus, genomic research is focused on the simpler *M. truncatula*, with the hope that genetic improvements to *M. sativa* will follow. The conference will compare and contrast the two species to help understand the limits of information transfer



between the two species. In addition, information developed on the *Medicago* system will be of value as a model for the development of tolerance in other crop systems, especially legumes, and for understanding the hierarchical and structure-function linkages in plants in general.

(2) Recent Similar Meetings

We know of no recent meetings that focused on the integration of plant protection, crop physiology, and *Medicago* genomics. Recent meetings on *Medicago* genomics have been held, such as "*Medicago* Genomics: New Tools for Legume Biology and Breeding", held at Toulouse, France in November, 2003 (http://medicago.toulouse.infr.fr/ EU/mtindex.htm). In the US, the first *Medicago* genomic meeting was "The Alfalfa Genome", held at the University of Wisconsin in August, 1999 (http://www.naaic.org/TAG/index.html). In addition, several workshops have been held on *Medicago trucatula* as a model system for legume biology (e.g., one at University of Wisconsin in July, 2001; http://www.naaic.org/Mtruncatula/index.html). Although some of the discussion at these workshops has included plant pathogen-*Medicago* interactions, the focus was on molecular mechanisms and not on crop protection. Integration across pest disciplines (entomology and plant pathology) and crop disciplines (production, physiology, breeding, molecular biology) for *Medicago* has not been attempted.

(3) Organizing Committee

Letters are attached from each member of the Organizing Committee. The members are:

1. William Lamp, Associate Professor
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(4) Proposed Program

We propose to hold the conference at the Samuel Roberts Noble Foundation, Ardmore, Oklahoma, where much of the research on *Medicago* genomics has been centered. The Foundation has graciously agreed to host us for a 2½ day meeting on 25-27 October, 2005. We have planned for five half-day sessions, including the speakers listed below, but also with discussions to focus on conference goals. Indeed, no speakers are planned for the last day; the emphasis will be on integration and identification of research needs. Posters will be encouraged from all participants to help communicate current and potential research areas.

Session 1: "The Focus on *Medicago*"
Chaired by **Landon Rhodes**, Ohio State University, OH

C.J. Nelson; University of Missouri, Columbia, MO; "Importance of *Medicago* species and economic consequences of their pests"
Charles Brummer; Iowa State University, Ames, IA; "Overview of *Medicago* genomics"
Richard Berberet; Oklahoma State University, Stillwater, OK; "Insect pests of *Medicago*"
Fred Gray; University of Wyoming, Laramie, WY; "Pathogen pests of *Medicago*"

Session 2: "Tolerance and Host Plant Resistance"
Chaired by **Mark McCaslin**, Forage Genetics International, MN

David Miller; Pioneer, WI; "Breeding for disease resistance in alfalfa"
Mark Sulc; Ohio State University, Columbus, OH; "Breeding for insect resistance in alfalfa"
Donald Miller; ABI, ID; "Breeding for nematode resistance in alfalfa"
George Vandemark; USDA-ARS, Prosser, WA; "Potential use of genomics in developing tools for pest resistance breeding"

Session 3: "Functional Genomics Research of *Medicago*"
Chaired by **Joseph Bouton**, Noble Foundation

Nevin Young; University of Minnesota, St. Paul, MN; "Update on *Medicago* genome sequencing project"
Doug Cook; University of California, Davis, CA; "*Medicago truncatula* to *Medicago sativa*: Use of the model system for alfalfa improvement"
Rick Dixon and **Lloyd Sumner**; Noble Foundation, Ardmore, OK; "Proteomic and metabolomic engineering in *Medicago*"
Deborah Samac; USDA-ARS, University of Minnesota, St. Paul, MN; "Using functional genomics in alfalfa improvement for pest resistance"

Session 4: "Physiology and Biochemistry of Biotic Stress Tolerance"
Chaired by **Jeffrey Volenec**, Purdue University

Jean-Christophe Avice; Universite de Caen, France; "Alfalfa stress tolerance mechanisms"
John Trumble; University of California, Riverside, CA; "Tolerance to biotic versus abiotic stresses"
Leon Higley; University of Nebraska, Lincoln, NE; "Stress tolerance to insect injury in legumes"
Yves Castonguay; Agriculture et Agroalimentaire Canada; "Physiology and biochemistry of alfalfa disease resistance"
Stephen Temple; Forage Genetics International, WI; "Biochemical targets for improved biotic stress tolerance in alfalfa"

Session 5: "Integration of Functional Genomics, Physiology and Biochemistry, and Tolerance from Biotic Stress in *Medicago*"
A discussion chaired by **William Lamp**, University of Maryland

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The expected outcomes of the conference are:

1. Presentations and minutes of the meeting will be made available at a website shortly following the meeting.
2. A book will be edited, including chapters by speakers and invited chapters from others, as well as a chapter with conclusions from the conference.
3. A new USDA multistate project proposal will be developed by NCT-205, to incorporate at least some of the needed research on biotic stresses on forage legumes.
4. New collaborations among scientists will be initiated, and previous collaborations will be strengthened, leading to new research proposals that build on existing information and tools, and that are focused on key research needs.

(5) **Method of Announcement**

We will employ various IPM and regional project listserves, and advertise through relevant professional societies. In addition, the Samuel Roberts Noble Foundation will establish a website with information about the conference.

(6) **References Cited**

Avice J.C., A. Ourry, G. Lemaire, and J. Boucaud. 1996. Nitrogen and carbon flows estimated by ^{15}N and ^{13}C pulse-chase labeling during regrowth of alfalfa. Plant Physiol. 112:281-290.

Baldwin, I.T., and C.A. Preston. 1999. The eco-physiological complexity of plant responses to insect herbivores. Planta 208: 137-145.

Beuselinck, P.R., J.H. Bouton, W.O. Lamp, A.G. Matches, M.H. McCaslin, C.J. Nelson, L.H. Rhodes, C.C. Sheaffer, and J.J. Volenec. 1994. Improving legume persistence in forage crop systems. J. Prod. Agric. 7: 311-322.

Bottrell, D.G. 1979. Integrated Pest Management Council on Environmental Quality. Washington, D.C. 120 pp.

Broscious, S.C., J.K. Pataky, and H.W. Kirby. 1987. Quantitative relationships between yield and foliar diseases of alfalfa. Phytopathology 77: 887-892.

Cate, J.R., and M.K. Hinkle. 1994. Integrated pest management: the path of a paradigm. National Audubon Society. Weadon Printing, Inc. Alexandria, Virginia.

Cook, D.R. 1999. *Medicago truncatula* – a model in the making. Curr. Opin. Plant Bio. 2: 30.

Elden, T.C., and M. McCaslin. 1997. Potato leafhopper (Homoptera: Cicadellidae) resistance in perennial glandular-haired alfalfa clones. J. Econ. Entomol. 90: 842-847.

Frisbie, R.E., and J.W. Smith, Jr. 1989. Biologically intensive integrated pest management: The future. In Progress and Perspectives for the 21st Century, J.L. Menn and A.L. Steinhauer, eds. Entomol. Soc. Amer., Lanham, MD.

Gibson, G. 2002. Microarrays in ecology and evolution: a preview. Mol. Ecol. 11: 17-24.

Graber, L.F., N.T. Nelson, W.T. Luekel, and W.B. Albert. 1927. Organic food reserves in relation to growth of alfalfa and other perennial herbaceous plants. Research Bull. 80, Univ. of Wis-Madison, USA.

Haagenson, D.M., S.M. Cunningham, B.C. Joern, and J.J. Volenec. 2003. Autumn defoliation effects on alfalfa survival, root physiology, and gene expression. Crop Sci. 43: 1340-1348.

Hansen, J.L., J.E. Miller-Garvin, J.K. Waldron, and D.R. Viands. 2002. Comparison of potato leafhopper-resistant and susceptible alfalfa in New York. Crop Sci. 42: 1155-1163.

Hanson, A.A., D.K. Barnes, and R.R. Hill, Jr. 1988. Alfalfa and Alfalfa Improvement. ASA-CSSA-SSSA Agronomy Number 29, Madison, Wisconsin.

Lamp, W.O., G.R. Nielsen, C.B. Fuentes, and B. Quebedeaux. 2004. Feeding site preference of potato leafhopper (Homoptera: Cicadellidae) on alfalfa and its effect on photosynthesis. J. Agric. Urban Entomol. (in press).

Lefko, S.A., L.P. Pedigo, and M.E. Rice. 2000. Symptoms and growth of potato leafhopper-tolerant alfalfa in response to potato leafhopper feeding. Agon. J. 92: 726-732.

Lewis, W.J., J.C. van Lenteren, S.C. Phatak, and J.H. Tumlinson, III. 1997. A total system approach to sustainable pest management. Proc. Natl. Acad. Sci. 94: 12243-12248.

Meuriot, F., J.C. Avice, M.L. Decau, J.C. Simon, P. Laine, J.J. Volenec, and A. Ourry. 2003. Accumulation of N reserves and vegetative storage protein (VSP) in taproots of non-nodulated alfalfa (*Medicago sativa* L.) are affected by mineral N availability. Plant Sci. 165: 853-863.

National Research Council. 1996. Ecologically Based Pest Management: new solutions for a new century. National Academy Press. Washington D.C. p. 115.

Noble Foundation, Samuel Roberts. 2004. http://www.noble.org.

Nutter, F. W., Jr., J. Guan, A.R. Gotlieb, L.H. Rhodes, C.R. Grau, and R.M. Sulc. 2002. Quantifying alfalfa yield losses caused by foliar diseases in Iowa, Ohio, Wisconsin, and Vermont. Plant Disease 86:269-277.

Painter, R. 1958. Resistance of plants to insects. Annu. Rev. Entomol. 3: 267-290.

Pennypacker, B.W., D.P. Knievel, K.T. Leath, E.J. Pell, and R.R. Hill, Jr. 1990. Analysis of photosynthesis in resistant and susceptible alfalfa clones infected with *Verticillium albo-atrum*. Phytopathol. 80: 1300-1306.

Peterson, R.K.D., and L.G. Higley. 2001. Illuminating the black box: the relationship between injury and yield. Pp. 1-12, in R.K.D. Peterson and L.G. Higley (eds.), Biotic Stress and Yield Loss. CRC Press, Boca Raton, FL.

Poston, F.L., L.P. Pedigo, and S.M. Welch. 1983. Economic injury levels: reality and practicality. Bull. Entomol. Soc. Am. 29: 49-53.

Rhodes, L. H. and Myers, D. K. 1986. Severity of spring black stem on alfalfa cultivars in Ohio. Plant Disease 70:746-748.

Smith, D. 1962. Carbohydrate root reserves in alfalfa, red clover, and birdsfoot trefoil under several management schedules. Crop Sci. 2:75-78.

Stowe, K.A., R.J. Marquis, C.G. Hochwender, and E.L. Simms. 2000. The evolutionary ecology of tolerance to consumer damage. Annu. Rev. Ecol. Syst. 31: 565-595.

Stuteville, D.L., and D.C. Erwin (eds.). 1990. Compendium of Alfalfa Diseases, 2nd ed. Amer. Phytopath. Soc. Press, St. Paul, MN.

Sulc, R.M., and W.O. Lamp. Insect Management. In Forages, vol. 2, R.F. Barnes, C.J. Nelson, M. Collins, and K.J. Moore (eds.). Iowa State Press, Ames, IA. *In press*

Sulc, R.M., and L.H. Rhodes. 1997. Planting date, fungicide, and cultivar effects on Sclerotinia crown and stem rot severity in alfalfa. Plant Disease 81:13-17.

Trumble, J.T., D.M. Kolodny-Hirsch, and I.P. Ting. 1993. Plant compensation for arthropod herbivory. Annu. Rev. Entomol. 38: 93-119.

U.S. Congress. 1995. Office of Technology Assessment, Biologically Based Technologies for Pest Control, OTA-ENV-636. Washington, D.C.

Volenec, J.J., A. Ourry, and B.C. Joern. 1996. A role for nitrogen reserves in forage regrowth and stress tolerance. Physiol. Plantarum 97:185-193.

Volenec, J.J., S.M. Cunningham, D.M. Haagenson, W.K. Berg, B.C. Joern, and D.W. Wiersma. 2002. Physiological genetics of alfalfa improvement: past failures and future prospects. Field Crops Res. 75: 97-110.

Wang, X., X. Ren, L. Zhu, and G. He. 2004. OsBi1, a rice gene, encodes a novel protein with a CBS-like domain and its expression is induced in responses to herbivore feeding. Plant Sci. 166: 1581-1588.