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# Research Uses Biotechnology In Search for Nematode Resistance

Suddenly, we have an efficient method for introducing foreign genes into soybean plants and testing them for their ability to confer nematode resistance

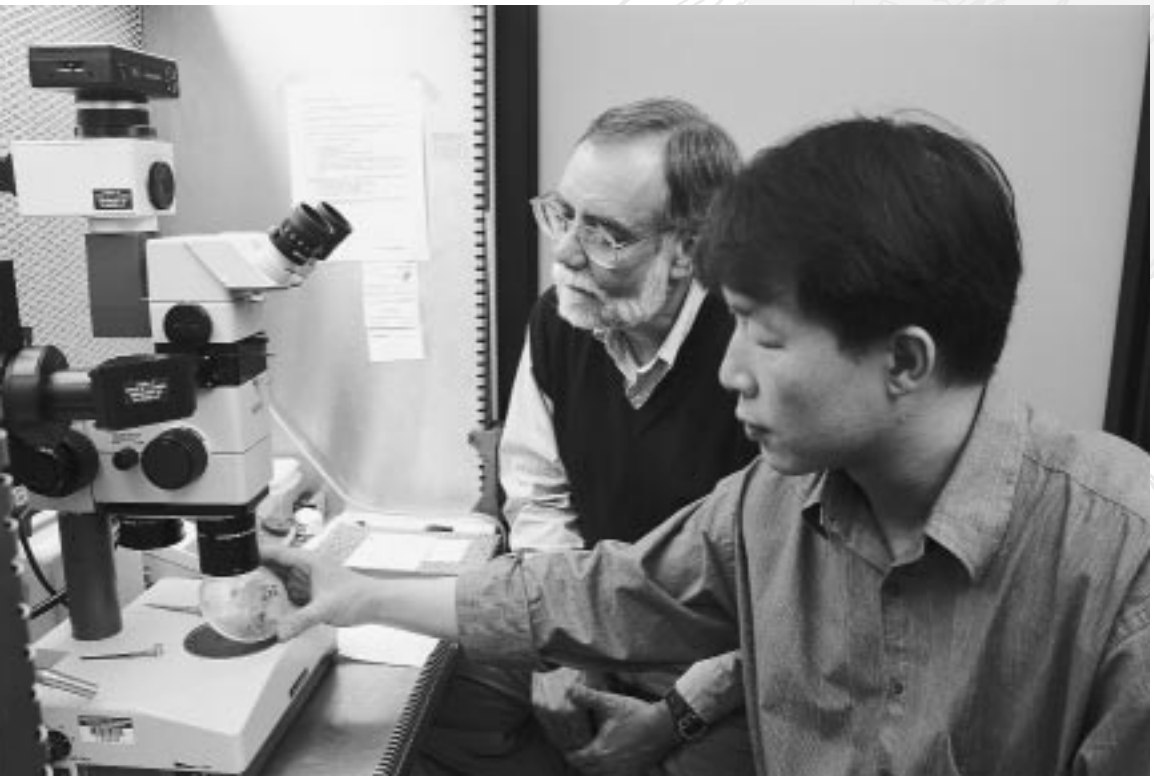
Soybean cyst nematode (SCN) ranks as the number one pest of soybeans with yearly losses in Illinois alone estimated as high as $120 million. The SCN problem has persisted across much of the Midwest despite significant efforts to develop resistant varieties from soybean germplasm using classical breeding techniques.

But, in research underway at the University of Illinois, scientists are now expanding the search for SCN-resistant genes to a wide range of other sources by applying the techniques of biotechnology.

"The traditional way of making soybean plants with resistance or tolerance to SCN is to identify genes in populations of soybeans and to breed these genes into favorable varieties," says Stephen Farrand, professor of molecular biology in the U of I's Department of Crop Sciences. "What we are trying to do is to use the new tricks of biotechnology to look for genes from sources such as bacteria or other species of plants that can be put into the soybean to confer unique types of resistance to SCN."

This project is funded by the Illinois Soybean Checkoff Board in collaboration with the United Soybean Board. Along with Farrand, the U of I research team includes plant physiologist Jack Widholm, USDA nematologist Greg Noel, and post-doctoral research associate Hyeon-Je Cho.

Farrand notes that the first step is to identify genes of interest that might have an effect on infection by SCN. The principle tool in this process is the bacterium known as *Agrobacterium rhizogenes*.



Stephen Farrand (left), professor of molecular biology in the Department of Crop Sciences, and Hyeon-Je Cho, post doctoral research associate, use a fluorescence dissecting microscope to examine soybean "fuzzy roots" that have been genetically modified. The target genes are inserted with a marker that allows them to be easily screened for resistance to soybean cyst nematode.

The biggest advantage for this technique, however, is that researchers are no longer restricted to soybean germplasm as the source of new genes

"This is one of a group of bacteria that have become the plant genetic engineer's favorite tools because they know how to send any DNA to plants," he says. "The DNA gets integrated directly into the plant and, if the DNA contains a properly constructed gene, the plant will express the trait in which we are interested. What's unique about *Agrobacterium rhizogenes* is that it causes plants to produce transformed roots—called hairy roots—that contain a piece of bacterial DNA."

These so-called hairy roots can be cut from the plant and grown virtually forever in an artificial medium in the laboratory.

"We know that we can use this technique to get nice hairy roots on soybeans and that we can grow the nematodes on these cultured roots," Farrand says. "This allows us to grow nematodes on the roots in the laboratory instead of on plants in the greenhouse."

The use of *Agrobacterium rhizogenes* provides an additional advantage because the bacterium can transfer a second gene along with the gene that causes the hairy roots.

"Suddenly, we have an efficient method for introducing foreign genes into soybean plants and testing them for their ability to confer nematode resistance." Farrand says.

As part of the project, Widholm is working to identify and clone foreign genes of interest. The researchers are looking at a number of different types of genes in that process.

"For example, much of the outer layer of a nematode is made up of complex chemicals, including collagen," Farrand says. "Many bacteria make enzymes that digest these products. Putting genes for these enzymes into the soybean could confer a potent form of resistance."

He explains that the new system allows researchers to screen through these genes to identify those that work best without the worry of having to develop a transgenic soybean plant at that stage of the project. It also lets the researchers focus on the root, which is the site of SCN infection.

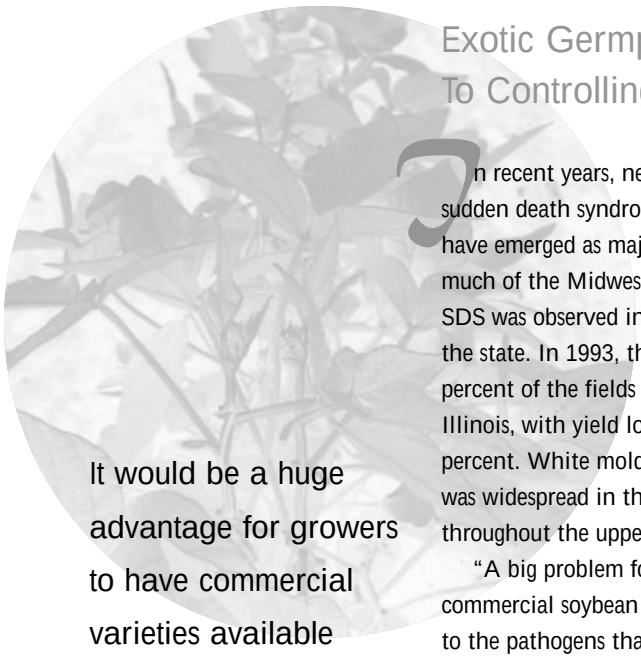
"Another major advantage is that the types of genes we are looking at are not race specific," Farrand says. "If you put a collagenase gene in the root and it affects the outer layer of a nematode, then it would not make any difference which race the nematode came from."

The system also has the advantage of avoiding many of the concerns expressed by opponents of transgenic food plants.

"Because SCN infects roots, it is perfectly conceivable to place the resistance gene behind an on-off switch that is on only in the root," Farrand says. "The product of this gene would not show up in the seed. So the foreign gene product would be absent in that part of the soybean that is used for commercial purposes."

The biggest advantage for this technique, however, is that researchers are no longer restricted to soybean germplasm as the source of new genes. Nevertheless, once a candidate gene has been identified as useful, then researchers still face the hurdle of getting it into a transgenic soybean plant.

"Once that has been done, it will still be up to breeders to move that gene into cultivars with high yield and other disease or herbicide resistant traits," Farrand says. "Although these molecular techniques cannot supplant classical breeding, they can identify and provide the breeder with genes from a much wider range of sources."



# Exotic Germplasm Could Hold Key To Controlling Emerging Diseases

In recent years, new soybean diseases such as sudden death syndrome (SDS) and white mold have emerged as major problems in Illinois and much of the Midwest. During a survey in 1998, SDS was observed in 90 percent of the counties in the state. In 1993, the disease was reported in 43 percent of the fields in some parts of east central Illinois, with yield losses ranging from 20 to 46 percent. White mold or sclerotinia stem rot also was widespread in the northern half of Illinois and throughout the upper Midwest during the 1990s.

"A big problem for growers is that most of the commercial soybean varieties are highly susceptible to the pathogens that cause those diseases," says plant pathologist Glen Hartman of the USDA Agricultural Research Service. "Because of the narrow genetic base of commercially grown soybeans, the outbreaks of SDS and white mold in most popular soybean varieties are likely to continue. It would be a huge advantage for growers to have commercial varieties available with resistance to these emerging diseases."

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Hartman notes that only partial resistance to SDS and white mold has been reported in commercial varieties. All the varieties tested, including those with partial resistance, still have roots that are colonized by the fungus that causes the disease and exhibit at least some foliar symptoms.

"Many people really did not pay attention to this problem until recently because neither of those diseases were thought to be very important," Hartman says. "Now we are finding that the fungus that causes white mold is present in many more fields than we ever imagined. Likewise, it appears that the pathogen that causes SDS is in the soil in most fields and probably has been for a long time. When environmental conditions are right, both diseases can be quite severe."

In response to this emerging problem, Hartman has been collaborating on several research programs aimed at locating new sources of resistance. In one of the projects underway at the National Soybean Research Laboratory, he has been working with Randall Nelson, curator of the USDA Soybean Germplasm Collection at the University of Illinois, and U of I plant geneticist Theodore Hymowitz on screening accessions of the wild annual progenitor of the soybean, *Glycine soja*, for resistance to SDS, white mold, and other major diseases.

Primary funding for this research was provided by soybean growers through the Illinois Soybean Checkoff Board. Additional funding was provided by the Illinois Council on Agricultural Research (C-FAR) and the Agricultural Research Service.

"The wild annual soybeans, *G. soja*, have only been screened for a few economically important traits," Hartman says. "So far, there are only a few publicly released, specialty-use soybean cultivars containing known genetic material from the wild annual soybeans. Even though soybeans and the annual progenitor can be crossed rather easily, this unique collection of genetic material has been virtually overlooked."

To date, the research team has screened more than 1,100 accessions of *G. soja* for resistance to the pathogen that causes SDS. About 5 percent of the collection of wild annual relatives has been classified as resistant. The entire collection was also screened for resistance to phytophtphora root rot. Six accessions were rated as resistant to that disease, including two from maturity groups III and IV. Several of the most promising accessions already are being crossed with soybean for further field and genetic studies.

"From a soybean breeding point of view, the wild annual soybeans are excellent candidates for gene exchange with soybean," Hartman says. "The sources of resistance found in *G. soja* accessions potentially include new genes that can be introduced into commercial soybeans. The release of this new germplasm could impact most breeding programs and provide soybean growers with new resistant varieties that would reduce losses due to SDS."

As part of this overall effort to combat emerging diseases, Hartman also has been working with Hymowitz on screening the wild perennial relatives of the soybean from the subgenus *Glycine* for resistance to both SDS and white mold. The subgenus contains 16 wild perennial species. These plants are indigenous to Australia and have been collected from other diverse environmental areas in southeast Asia and the South Pacific.

Major funding for the screening efforts came from the Agricultural Research Service and from the U of I Experiment Station.

"The perennial *Glycine* species represent a virtually untapped resource of potentially novel genes for improving traits in soybeans, including disease resistance," Hartman says. "We have already screened more than 1,000 of the perennial accessions. There are numerous examples where wild relatives of a cultivated crop have provided unique traits, including disease resistance. That scenario could well be repeated for soybeans."

Initial evaluations of the perennial species for disease resistance have provided a number of promising resistant accessions that will be further tested in the field during the coming growing season. The researchers also are searching this collection for resistance to other important diseases, such as brown stem rot and charcoal rot. Future plans call for undertaking the complicated crosses with commercial soybeans for the most promising genetic material.

"These resistant accessions probably represent a totally different gene pool than is present in our commercial soybean varieties," Hartman says. "For the perennials, in particular, it is important to realize that this screening is just the first step. A lot of field testing will be needed even after we complete the difficult task of crossing them with soybeans. Nevertheless, this material could well represent what soybean growers will be using in their fields in the years to come."

According to Hartman, the genetic material from the wild annual relatives rather than the perennial species almost certainly will have a more immediate impact in breeding programs, although both hold major promise for the future. He further adds that this research project is only part of a larger effort that has just begun to unravel many of the mysteries of these emerging diseases.

"Often times people expect nothing less than a silver bullet from this kind of research," he says. "It is important to realize that we have had ongoing research on SDS for only a relatively short time—four or five years. For white mold, it has been even a shorter time. Nevertheless, what's encouraging is that we already have been able to discover a lot of valuable information on these diseases that will benefit soybean growers far into the future."

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## Soy Moves to the Front Line in the War on Heart Disease

Despite persistent warnings about the need for changes in the average American diet, more than 750,000 people die each year in the United States from coronary heart disease for which moderate to high cholesterol levels are a contributing cause. At least 20 percent of adults have blood cholesterol levels greater than 240 milligrams per deciliter (mg/dL). Another 31 percent fall into the borderline-high category with cholesterol levels over 200 mg/dL.

The U.S. Food and Drug Administration (FDA) estimates that 52 million Americans over the age of 20 are candidates for some type of dietary or drug intervention to lower blood cholesterol. Today, however, it is becoming clear that increased consumption of soy protein offers one of the most promising ways to lower blood cholesterol levels without the use of drugs.

In 1995, a comprehensive review, or meta analysis, of 29 previously published studies, including two from the University of Illinois, confirmed that an average of 47 grams of soy protein per day can produce significant decreases in total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides, without affecting desirable high-density (HDL) cholesterol levels.

"Until that meta analysis took place, few health care professionals noticed that there was a lot of support in the published literature for the idea that soy protein was a dietary factor that could lower cholesterol," says John W. Erdman, Jr., director of the U of I's Division of Nutritional Sciences. "The effect goes beyond the impact of lower fat and cholesterol intake. It is a direct impact of soy protein."

Most recently, Erdman has collaborated on a major new study measuring the long-term effects of soy protein on cholesterol levels in post-menopausal women. Other researchers in the

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John W. Erdman, Jr. (Left), director of the Division of Nutritional Sciences at the U of I, observes as graduate assistant Christine Atkinson prepares a blood serum sample for a cholesterol study on subjects eating soy products. This research has resulted in a proposed rule that would authorize the use of health claims on food labels linking soy protein with reduced risk of heart disease.

study were from the Division of Nutritional Sciences, the Department of Food Science and Human Nutrition, and the Department of Veterinary Pathobiology at the University of Illinois at Urbana-Champaign and the Division of Epidemiology and Biostatistics at the University of Illinois at Chicago.

In this research, post-menopausal women who had slightly high cholesterol levels received a milk-based supplement with 40 grams of soy protein a day or one of two soy supplements in the form of isolated soy protein. They received this diet for six months and were measured for total cholesterol and for LDL and HDL levels. Primary funding for the study came from the Illinois Soybean Checkoff Board, with additional support from Protein Technologies International, a division of Dupont.

"The bottom line from the study was that LDL—the bad cholesterol—went down, while HDL cholesterol—the good stuff—went up in both the soy groups in the study," Erdman says.

He emphasizes that these findings are especially important because few studies have evaluated women specifically, and post-menopausal women have never been distinguished from women in general, even though heart disease is a leading cause of death in older women.

"Not only were the changes statistically significant, they were maintained over the whole six-month period," Erdman says. "What makes that even more important is the fact that this was the longest study that's every been published on soy."

In another study funded by the Illinois Council on Food and Agricultural Research (C-FAR), Erdman and other researchers have been examining the impact of the level of soy protein consumption on cholesterol lowering. This study focused on men with mildly high cholesterol levels. They were fed diets with 20, 30, 40 or 50 grams of soy protein a day for six weeks.

"Initial evaluation of the results suggests that as little as 20 grams of soy protein—which you certainly can get in one or two servings of soy foods—could reduce cholesterol in this group,' Erdman says. "If this data is confirmed, we will be able to show that just a moderate alteration in the diet can result in a significant reduction in serum cholesterol."

The results of this study will be especially relevant because the Food and Drug Administration has proposed a new rule that would authorize the use of health claims on food labels linking soy protein with reduced risk of heart disease. The agency has tentatively concluded from the scientific evidence that 25 grams of soy protein included in a daily diet that is low in saturated fat and cholesterol may reduce the risk.

"Acceptance of this food labeling rule would be recognition that soy protein is, in fact, important in lowering cholesterol," Erdman says. "It also could be a big boon to soybean farmers as more and more companies would have the incentive to incorporate soy at higher levels in their food products."

If finalized, this new rule would allow for labeling on food products that contain at least 6.25 grams of soy protein in a single serving. Four servings a day would make up the recommended daily minimum intake of 25 grams. Most of the traditional soy foods, such as tofu, would meet the labeling requirements.

Erdman also expects that FDA approval would have a major impact on the soybean industry by spurring commercial manufacturers to develop many new soy food products.

"What's so exciting to me personally is that much of the research on which that proposed rule is based was conducted at the U of I," Erdman says. "This is the culmination of an effort that has been going on since the 1980s. The soybean farmers through the Checkoff Board have had faith in us and supported this work all along. It's taken some years, but we now can see a real outcome from all that support."

# U of I Project Aims to Overcome Consumer Resistance to Soy Foods

Although new research has confirmed many health benefits from eating soy foods, per capita consumption of soy has not increased as much as would be expected. In fact, consumer resistance continues to be a stumbling block in expanding the market for many new soy products. Yet, according to Brian Wansink, associate professor of business administration and agricultural and consumer economics at the University of Illinois, there are numerous behavioral, demographic, and psychological differences that can clearly distinguish frequent consumers of soy from infrequent users.

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"Our goal is to examine what influences the acceptance and consumption of soy-based foods," Wansink says. "In particular, we want to understand more about those who consume or do not consume soybeans. By better understanding how to communicate the health-related benefits of these foods and simultaneously counter the perceived taste-related drawbacks, we hope to increase the acceptance of soy among larger segments of the population. Ultimately that will help expand the markets for a major Illinois farm product, while improving the health and nutrition of consumers."

Wansink serves as the director of the U of I's Food and Brand Lab, which consists of a series of test kitchens and cooperating grocery stores that are used to understand how consumers choose and use foods and packaged goods. In collaboration with researchers from fields as diverse as cultural anthropology and agricultural and consumer economics, the mission of the Food and Brands Lab supports ongoing projects on packaging, promotions, and consumer usage and acceptance of products.

With support from the Illinois Council on Food and Agricultural Research (C-FAR) and the Illinois Soybean Checkoff Board, he is working in collaboration with NSRL Director Steve Sonka and Marketing Professor Michelle Morganosky on several projects directly aimed at promoting the acceptance of soy products among consumers.

"A major component initially is to look at a large group of consumers to determine what distinguishes people who would be predisposed to eat soy," Wansink says. "We are not simply going to focus on people who are interested in health or who are vegetarians. The idea is to find out what sort of messages, products, and delivery systems are going to be most effective in targeting a number of other specific groups."

In one part of the study, a large number of Asian students were interviewed to determine what it was they liked about soy. Results indicated that there were two distinct groups: those who eat soy for health reasons and those who eat soy because they like the taste.

"What we found out was that those who liked the taste shared the characteristic that they lived in a household with someone who is a very good cook," Wansink says. "The most powerful thing that result suggests is that liking the taste of soy can be learned."

A second study has been looking at the different perceptions of soy among a group of about 80 home economics teachers. The study showed that perceptions of soy grouped into four major clusters. The heaviest users of soy in the study were those who were lactose intolerant.

"In Asia, we have people who like soy for health reasons and for taste," he says. "What we find among the home economists is that many people who know a lot about soy are those who use it because of dietary restrictions."

Other studies clearly showed that simply listing soy as an ingredient negatively influenced people's perceptions in regard to taste, even if the product contained no soy. At the same time, health claims mentioned in association with soy became believable to consumers. In fact, there was a powerful finding of deference by consumers to major health claims about soy in general.

"Based on those studies, a health label should certainly be used along with a soy ingredient label to diminish the negative taste perceptions," Wansink says. "Advertisers and media planners with products that are labeled as soy should be sure to target dieters and health-conscious consumers. The soy ingredient labels should not be used for taste-conscious consumers."

The research further revealed that the most effective way to present health claims for soy was to have a label on the front with a brief statement of the claim and a more detailed explanation on the back of the package. Results clearly indicated that this combination provided the best way to get the health message across to consumers.

"The short claim on the front improves their understanding, but the long claim on the back makes them more willing to believe it," he says. "It's one thing to understand something but it is another thing altogether to actually believe it. That's what is so powerful about this combination of labels."

A key component of this research will focus on disseminating the data as widely as possible, especially to dieticians, health care professionals, and media. As part of that effort, Wansink will feature many of the results in special programs at Global Soy Forum '99 in Chicago from August 4 to 7.

"The most interesting results will center on how we can identify what sort of soy products in a highly processed form will be successful," Wansink says. "It will take forever to get many people to eat tofu, but it is not that big a stretch to get them to eat vegetarian soy chili, for example. That is where the lowest hanging fruit is to be found."

Further details about the research on soy foods and the Food and Brand Lab are available on the internet at http://www.foodandbrandlab.com or www.consumerpsychology.com.

## From the Director's Desk

The date for Global Soy Forum '99 is rapidly approaching. This groundbreaking event, which includes the Sixth World Soybean Research Conference, will be held in Chicago from August 4 to 7, 1999. Of course, we all are quite excited about the support we have received in planning this event and the interest so many people have expressed about attending.

As NSRL Director, I have the exciting opportunity of interacting with users of soy research, public and private sector constituents who support soy research, and soy researchers themselves. The diverse program planned for Global Soy Forum '99 clearly reflects the fact that today is a tremendously interesting time to be involved in the world of soy research. The opportunities and the challenges we face certainly are daunting and complex. At the same time, the potentials for social gain and personal fulfillment are equally significant. A central feature of the future soy research system is that researchers will need both to master the detailed complexity of their scientific specialty and to increase their knowledge of how their area of expertise interacts within the broader soy industry complex.

Global Soy Forum '99 has been designed with those challenges in mind. In fact, all soy decision makers, not just researchers alone, require detailed expertise regarding specific components of the system and considerable understanding of how the entire system works. That is why Global Soy Forum '99 encompasses more than just one set of presentations and papers.

Examination and dialogue regarding the forces that will determine future demand and supply in the world are central to Global Soy Forum '99. Critical forces such as population growth, trade, technology and societal response to technology, new opportunities for soy foods, and structural change in agriculture, also will be emphasized. In addition, Global Soy Forum '99 will integrate two successful and established meetings with three innovative, first-time initiatives:

- World Soybean Research Conference VI—an international meeting of more than 600 soybean scientists.
- Midwest Soybean Conference 4—an annual conference focusing on producer-oriented topics.
- Soy in Animal Nutrition Symposium—a research conference featuring the latest developments in the use of soy in animal feeds.
- Focus on Soy Foods—the emerging role of soy foods will be featured during the entire event, including the Soy Symposium, which will be an all-day event sponsored by the United Soybean Board.
- Knowledge Management Center—a forum for providing hands-on experience with the latest developments in information technology applied to the soybean industry.

Global Soy Forum '99 will provide participants with the opportunity to become more knowledgeable about the key strategic issues shaping the future of agriculture, while expanding their detailed knowledge in specific areas of work and expertise. Of course, that result is far from easy to accomplish. It is, however, the task that all of us in the sector must strive to achieve if we are to respond to the challenges and opportunities of the future world of soybeans.

More specific information about the event's program, affiliated activities, and registration procedures can be found at http://www.gsf99.uiuc.edu or by contacting me at the NSRL mailing address included in this newsletter or by e-mail at nsrl@uiuc.edu.

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In this issue



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