ISB NEWS REPORT
AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY

January 2015

RISK ASSESSMENT

CONTENTS



Virginia Tech
Invent the Future
Virginia Polytechnic Institute and State University

# Crop Diversity Delays Evolution of Insect Resistance to Bt Cotton in China

*Bruce E. Tabashnik and Yidong Wu*

## Background

To protect crops from insect pests, scientists have genetically engineered cotton, corn and soybean to produce insecticidal proteins from the common bacterium *Bacillus thuringiensis* (Bt)[1,2]. These environmentally friendly Bt toxins kill some of the world's most damaging crop pests such as *Helicoverpa armigera* (cotton bollworm), but are harmless to most other species including humans[1,3]. Organic growers and others have used Bt toxins in sprays for more than 50 years and mainstream farmers have planted transgenic Bt crops since 1996.

During 2013, farmers worldwide grew 190 million acres of Bt cotton, Bt corn, and Bt soybean[2]. In the United States, Bt corn accounted for 80% of all corn and Bt cotton accounted for 84% of all cotton planted in 2014 (ref. 4). In China, the world's leading cotton producer, Bt cotton is the only Bt crop grown widely. In northern China, where most of the nation's cotton is grown, Bt cotton has accounted for 98% of cotton planted since 2010 (ref. 5).

Although Bt crops have helped to suppress pests, decrease insecticide sprays, and promote biological control by natural enemies[6-9], rapid evolution of resistance to Bt toxins by pests can diminish or even eliminate these benefits[10]. The primary strategy for delaying pest resistance to Bt crops aims to increase the survival of toxin-susceptible insects with 'refuges' of plants that do not produce Bt toxins and can serve as hosts for the pests[10]. In the United States, Australia, and most other countries, farmers were required to plant refuges of non-Bt cotton near the first type of Bt cotton that was commercialized, which produces one Bt toxin named Cry1Ac. In the United States and Australia, farmers have switched to Bt cotton that produces two different toxins[10].

In China, however, farmers still plant Bt cotton producing only Cry1Ac and refuges of non-Bt cotton have not been required. The approach adopted in China is based on the hypothesis that abundant non-Bt host plants other than cotton (such as non-Bt corn, soybean, peanut, and other crops) provide sufficient 'natural refuges' to delay the evolution of resistance in the *H. armigera*[11]. Factors thought to favor success of the natural refuge strategy for *H. armigera* in northern China include the high proportion of non-Bt host plants, the close proximity of small plantings of cotton and non-cotton host plants, and this pest's high mobility and extensive gene flow. Understanding the effects of the natural refuge strategy on *H. armigera* has broad implications because this species is one of the most important and widespread pests targeted by Bt crops worldwide.

Initial work on the natural refuge strategy for Bt crops included indirect investigation of its potential to delay resistance[11] and qualitative comparisons of its effects on field-evolved resistance between populations of *Helicoverpa zea* and among species including *H. armigera*[12]. Nonetheless, despite implementation of this strategy for delaying resistance to one-toxin Bt

PUBLISHED BY

**Information Systems for Biotechnology**

Virginia Tech
1900 Kraft Drive, Suite 103
Blacksburg, VA 24061

Tel. 540-231-3747
Fax 540-231-4434

Subscribe to and access archived issues of the ISB News Report online:

**www.isb.vt.edu**

Editor:
Ruth Irwin
rirwin@vt.edu

ISB welcomes your comments and encourages article submissions. If you have a suitable article relevant to our coverage of agricultural and environmental applications of genetic engineering, please email it to the Editor for consideration.



cotton in China since 1997 and for two-toxin Bt cotton in the United States since 2007 (ref. 13), no rigorous large-scale tests of this strategy had been reported previously.

Two conditions in northern China during 2010 to 2013 provided an exceptional opportunity for testing the natural refuge strategy. First, in each of the six provinces studied during this period, Bt cotton accounted for >94% to 100% of all cotton, which limited the contribution of non-Bt cotton to any observed delays in resistance. Second, almost 1% of *H. armigera* individuals were resistant to Cry1Ac in northern China in 2010 (ref. 14), so without effective refuges, a large increase in resistance would be expected by 2013. We tested the natural refuge strategy with a synergistic analysis combining computer simulation modeling with field monitoring data for *H. armigera* based on multi-generational evaluation of resistance to Cry1Ac in 46 sets of insects collected during 2010 to 2013 from six provinces in northern China (n = 70,916 larvae tested from all sources)[5].

## Predicted Outcomes from Simulations

We conducted simulations of a population genetic model to project the consequences of different hypotheses about the effects of natural refuges on the evolution of resistance to Cry1Ac by *H. armigera* in northern China from 2010 to 2013 (ref. 5). We based key parameters in the model on empirical data for *H. armigera* in northern China, using previously published data and obtaining new data as needed. We set the percentage of resistant individuals in 2010 at 0.93%, which is the mean percentage of resistant individuals observed in northern China in 2010 (ref. 14). We varied assumptions about dominance in sensitivity analyses, using either a standard one-locus model with three levels of dominance, or a more realistic approach incorporating alleles at three independent loci that conferred recessive, dominant or additive inheritance of resistance.

To evaluate the hypothesis that natural refuges (non-Bt host plants other than cotton) do not contribute to delaying resistance, we simulated a 2% refuge because non-Bt cotton accounted for 2% of all cotton planted in northern China during 2010 to 2012 (ref. 5). The simulation results show that without natural refuges, the projected percentage of resistant individuals increased to >50% by 2011 and >98% by 2013 regardless of dominance (**Fig. 1**).

In striking contrast, under the hypothesis that natural refuges delay resistance, resistance evolved much slower (**Fig. 1**). In the most optimistic scenario, we modeled a 90% refuge because *H. armigera* host plants other than Bt cotton (non-Bt cotton, corn, peanut, and others) accounted for 90% of the total area of *H. armigera* host plants in northern China during 2010 to 2012. Thus, the 90% refuge reflects the assumption that each hectare of a non-Bt host plant was equivalent to a hectare of non-Bt cotton in terms of the contribution as a refuge. In this scenario, with alleles at three loci conferring resistance, the percentage of resistant individuals increased from the initial value of 0.93% in 2010 to only 1.1% in 2013.

In a more realistic scenario, we simulated a 56% 'effective refuge' based on the estimated contribution of susceptible *H. armigera* moths from non-Bt cotton and other non-Bt host plants. We estimated a 56% effective refuge by adjusting the area planted to non-Bt cotton and three other major types of non-Bt host plants (corn, peanut, and legumes other than peanut) in northern China for the observed number of *H. armigera*

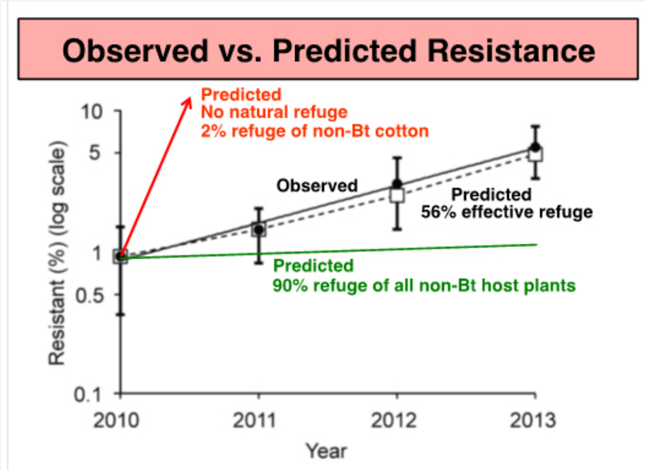


**Figure 1.** Observed versus predicted evolution of resistance to Bt cotton by *H. armigera* in northern China (adapted from ref. 5). The black circles show the observed mean percentage of resistant individuals based on survival in diagnostic concentration bioassays of $F_1$ progeny of field-collected insects (mean n = 11,178 larvae tested for each circle); bars show 95% confidence intervals. The solid black line from linear regression of the log-transformed observed means indicates a significant increase in resistance over time (slope = 0.26, $r^2 = 0.99$, df = 2, P = 0.005). Predicted outcomes are based on simulations of a population genetic model under three different assumptions about natural refuges: no natural refuge (red, 2% refuge of non-Bt cotton), contribution of susceptible moths per hectare is the same for natural refuges and non-Bt cotton (green, 90% refuge including all non-Bt host plants), and effective refuge percentage based on adjusting the contribution of susceptible moths for each hectare of non-Bt host plant relative to a hectare of non-Bt cotton (open squares connected by dashed black line, 56% effective refuge).

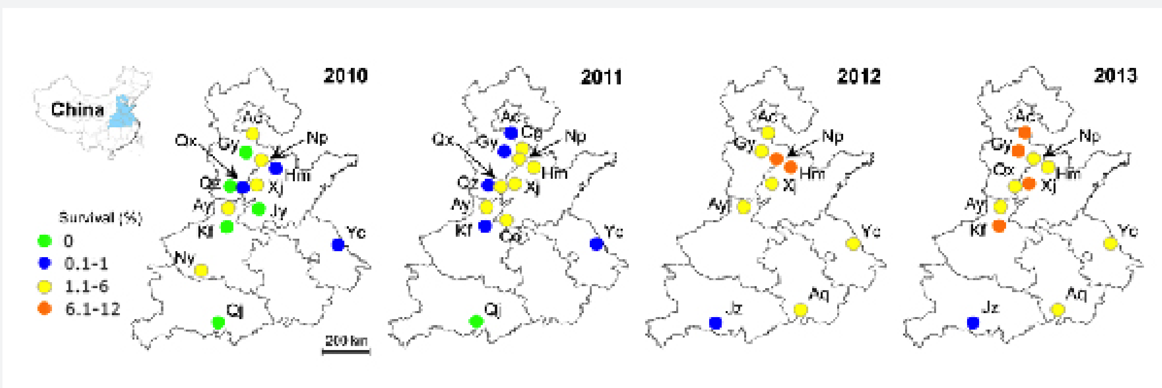


**Figure 2.** Survival at a diagnostic concentration of Cry1Ac for $F_1$ progeny of *H. armigera* from 17 sites in six provinces of northern China[5]. The percentage of resistant individuals increased from 0.93% in 2010 to 5.5% in 2013. Anhui province: Anqing (Aq). Hebei province: Anci (Ac), Cangxian (Cg), Gaoyang (Gy), Nanpi (Np), Qiuxian (Qx), and Quzhou (Qz). Henan province: Anyang (Ay), Kaifeng (Kf), and Nanyang (Ny). Hubei province: Jingzhou (Jz) and Qianjiang (Qj). Jiangsu province: Yancheng (Yc). Shandong province: Caoxian (Co), Huimin (Hm), Juye (Jy), and Xiajin (Xj). Each circle represents results from testing a mean of 972 larvae.

moths emerging per hectare from each of these non-Bt plants relative to non-Bt cotton in field experiments[5]. With a 56% refuge and alleles at three loci conferring resistance, the percentage of resistant individuals increased from 0.93% in 2010 to 4.9% in 2013 (**Fig. 1**).

## Observed vs Predicted Evolution of Resistance

Results from monitoring resistance to Cry1Ac in 17 field populations of *H. armigera* from 2010 to 2013 support the hypothesis that natural refuges delayed resistance. For all populations monitored in northern China, the mean percentage of resistant individuals (based on survival at a diagnostic concentration of 1 microgram of Cry1Ac per $cm^2$ diet) rose six-fold in three years: from 0.93% in 2010 to 5.5% in 2013 (**Figs. 1 & 2**). This observed increase in resistance corresponds closely with the increase predicted assuming a 56% effective refuge (4.9% resistant individuals in 2013, **Fig. 1**). The observed data do not fit well with the predictions assuming either no natural refuge (2% non-Bt cotton refuge only, > 98% resistant individuals predicted in 2013) or that each hectare of natural refuge is equivalent to a hectare of non-Bt cotton (90% refuge of non-Bt host plants, 1.1% resistant individuals predicted in 2013) (**Fig. 1**).

In contrast with the significant increases in resistance seen in northern China, survival at the diagnostic concentration was 0% in all four years in two field populations from areas in northwestern China where Bt cotton was not grown extensively (n = 5345) and in a susceptible lab strain (n = 2232).

## Conclusions

The results imply that the natural refuge consisting of non-Bt host plants other than cotton delayed resistance of *H. armigera* to Bt cotton in northern China. However, the monitoring data do show a six-fold increase in the percentage of resistant individuals in three years, from 0.93% in 2010 to 5.5% in 2013. If this trajectory continues, based on extrapolation of the linear regression of the observed data in **Fig. 1**, the percentage of resistant individuals will exceed 50% by 2017. The results from northern China also reinforce concerns about rapid evolution of *H. armigera* resistance to Bt crops in Brazil, where natural refuges are limited because farmers plant Bt corn and Bt soybean as well as Bt cotton[15]. Relative to northern China, where plantings of cotton and non-cotton host plants are small and close to each other, the success of natural strategy could be reduced in the United States and other places where cotton plantings are larger and farther from non-cotton host plants.

An immediate switch to two-toxin cotton producing Cry1Ac and Cry2Ab could be useful for slowing evolution of H. armigera resistance to Bt cotton in northern China because cross-resistance between these two toxins is limited. Because cross-resistance of H. armigera does not occur between Vip3A and either Cry1Ac or Cry2Ab16, Bt cotton producing these three toxins could be especially effective for suppressing resistance. This type of three-toxin Bt cotton may be available by 2016 (ref. 16). In addition to increasing the number and diversity of toxins in transgenic cotton, integration of Bt cotton with other tactics such as sterile releases or biological control could delay the evolution of resistance and provide a more sustainable pest management system[8,9].

**References**

1. Sanahuja G, Banakar R, Twyman RM, Capell T, Christou P (2011) *Bacillus thuringiensis*: a century of research, development and commercial applications. Plant Biotechnol J 9: 283-300.
2. James C (2013) Global status of commercialized Biotech/GM crops: 2011. ISAAA Brief No. 46. ISAAA, Ithaca, New York.
3. Mendelsohn M, Kough J, Vaituzis Z, Matthews K (2003) Are Bt crops safe? Nature Biotechnol 21: 1003-1009.
4. United States Department of Agriculture. Economic Research Service. Adoption of genetically engineered crops in the U.S. http://www.ers.usda.gov/Data/BiotechCrops/
5. Jin, L, Zhang H, Lu Y, Yang Y, Wu K, Tabashnik BE, Wu Y. 2014. Large-scale test of the natural refuge strategy for delaying insect resistance to transgenic Bt crops. Nature Biotechnol.: Published online 15 December 2014. doi:10.1038/nbt.3100.
6. Wu K, Lu Y, Feng H., Jiang Y, Zhao, JZ (2008) Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. Science 321:1676–1678 (2008).
7. Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW, Fleischer SJ, Abrahamson M, Hamilton KL, Steffey KL, Gray ME, Hellmich RL et al. 2010. Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science 330: 222-225.
8. Tabashnik BE, Sisterson MS, Ellsworth PC, Dennehy TJ, Antilla L, Liesner L, Whitlow M, Staten RT, Fabrick JA, Unnithan GC, Yelich AJ, Ellers-Kirk C, Harpold VS, Li X, Carrière Y (2010) Suppressing resistance to Bt cotton with sterile insect releases. Nature Biotechnol 28: 1304-1307.
9. Lu, Y, Wu K, Jiang Y., Guo Y, Desneux N, (2012) Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature 487: 362-367.
10. Tabashnik BE, Brévault T, Carrière Y (2013) Insect resistance to Bt crops: Lessons from the first billion acres. Nature Biotechnol 31: 510-521.
11. Wu K, Guo Y (2005) The evolution of cotton pest management practices in China. Annu Rev Entomol 50: 31-52.
12. Tabashnik BE., Gassmann AJ, Crowder DW, Carrière, Y. (2008) Insect resistance to Bt crops: evidence versus theory. Naure Biotechnol 26: 199-202.
13. US Environmental Protection Agency (2007) Pesticides news story: EPA approves natural refuges for insect resistance management in Bollgard II cotton. http://www.epa.gov/oppfead1/cb/csb_page/updates/2007/bollgard-cotton.htm
14. Zhang H, Yin W, Zhao J, Jin L, Yang Y, Wu S, Wu K, Cui J, Tabashnik BE, Wu Y (2011) Early warning of cotton bollworm resistance associated with intensive planting of Bt cotton in China. PLoS ONE 6(8): e22874. doi:10.1371/journal.pone.0022874.
15. Tay WT, Soria MF, Walsh T, Thomazoni D, Silvie P, Behere GT, Anderson C, Downes S. (2013) A brave new world for an Old World pest: *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil. PLoS ONE 8(11): e80134. doi:10.1371/journal.pone.0080134
16. Mahon R, Downes SJ, James B (2012) Vip3A resistance alleles exist at high levels in Australian targets before release of cotton expressing this toxin. PLoS ONE 7(6): e39192. doi:10.1371/journal.pone.0039192

*Bruce E. Tabashnik*
*Department of Entomology*
*University of Arizona*
*Tucson, AZ 85721, USA*
*brucet@cals.arizona.edu*

*Yidong Wu*
*Key Laboratory of Integrated Management of Crop Diseases and Pests College of Plant Protection*
*Nanjing Agricultural University*
*Nanjing 210095, China*
*wyd@njau.edu.cn*

# *Brachypodium distachyon* Provides Insights into Plant Trade-offs Between Growth and Stress Tolerance

*Antoine Peraldi, Rachel Goddard and Paul Nicholson*

## Current situation, needs and challenges for the future of cereal crop production

Cereals provide the vast majority of the food supply for humans and animals worldwide. The projected needs of an ever growing world population are threatened by several factors that negatively impact crop yield and quality. Diseases caused by viruses, bacteria, fungi, and oomycetes or damage caused by insect herbivores are commonly referred to as biotic stresses. Adverse environmental conditions such as droughts, flooding, and temperature extremes, as well as pollution of soils by toxic chemicals or excess salts are referred to as abiotic stresses. The development of crop varieties with improved tolerance to both biotic and abiotic stresses is critical to meet the challenges that lay ahead.

## Phytohormones regulate plant response to stresses

Plant hormones (phytohormones) are small signaling molecules that play a central role in orchestrating the complex regulatory mechanisms involved in plant development and in mounting appropriate responses to biotic and abiotic stresses. Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are hormones that have essential roles in plant immunity. SA-dependent defense mechanisms are mainly effective against biotrophic pathogens such as mildews and rusts. These pathogens feed from living host cells, making it essential that they maintain viability of the host. SA-mediated defense responses include the hypersensitive response (HR) that leads to death of the infected host cell and subsequent starvation or poisoning of the pathogen. The JA/ET mediated defenses are mainly induced against feeding insects and necrotrophic pathogens that kill host cells and feed on dead host tissues.

The SA and JA/ET signaling pathways generally function antagonistically, with activation of one leading to suppression of the other. Much of this antagonism is mediated by the regulatory protein NPR1. NPR1 binds directly to SA and is required for SA defense responses and suppression of JA response mechanisms. Mutants in NPR1 exhibit a reduction in SA mediated responses and an enhancement of JA-dependent defenses. The hormone ET has been shown to have a modulatory effect on both pathways, meaning that NPR1 is not essential for crosstalk between SA and JA[1].

This dichotomous understanding of the regulation of plant immunity has become increasingly challenged, and a far more complex situation has emerged in which extensive interconnection with other hormonal signals fine-tunes the outcome of plant-pathogen interactions. Other phytohormones such as auxins, cytokinins (CKs), and absiscic acid (ABA) also regulate a myriad of plant physiological mechanisms and stress-related responses, including abiotic stresses. Although gibberellic acid (GA) and brassinosteroids (BRs) are growth-promoting hormones involved in a plethora of biological processes vital for the development and reproduction of plants, recent research has revealed that they play important roles in regulating a trade-off between growth and immunity.

## GA regulates trade-offs between growth and stress tolerance

Growth is restrained by a family of proteins named DELLAs for the conserved amino-acid motif near their N-terminus. Accumulation of GA leads to degradation of the DELLA proteins in the so-called ‘relief of restraint’ model. Mutation of DELLA proteins leading to greater stabilization results in attenuated GA sensitivity, reduced growth, and shorter plant stature, even under optimal growth conditions. Introduction of reduced height (*Rht*) alleles in wheat, which encode stabilized forms of DELLA proteins, played a critical role in increasing crop yield during the so-called “Green Revolution,” as higher nitrogen inputs could be applied without plants growing too tall and falling over (lodging). Wheat and barley lines possessing stabilized DELLA proteins are hyposensitive to the GA signal. These lines show enhanced resistance towards necrotrophic pathogens but an increased susceptibility to biotrophic pathogens[2]. These findings demonstrate that GA signaling has a pleiotropic effect on plant growth and disease resistance.

As mentioned above, JA/ET signaling is involved in mediating resistance against necrotrophic pathogens. Recent studies provide insight into the potential mechanisms that link DELLAs to JA signaling and suggest a function for pathway control and signal integration[3]. DELLA proteins interact with JA ZIM-domain (JAZ) proteins, which are negative regulators of JA-responsive genes involved in disease resistance. In the absence of stressful conditions, GA is produced by the plant and DELLA proteins are degraded, therefore allowing the plant to grow. Under stressful conditions, in the absence of GA, DELLA proteins accumulate and compete with the JA transcription factor MYC2 for binding to JAZ proteins. This frees MYC2 to activate JA-responsive genes leading to enhanced resistance to necrotrophic pathogens.

It is assumed that due to the antagonistic nature of the JA-SA pathway the increase in JA signaling leads to suppressed SA-mediated responses and increased susceptibility to biotrophic pathogens. This hypothesis provides a molecular basis for trade-offs between resistance to pathogens of differing life-style. Exposure to abiotic stresses, such as high salinity and low temperature, leads to an accumulation of DELLA proteins and a reduction in growth. DELLA proteins have a role in promoting cell survival by reducing the accumulation of reactive oxygen species (ROS) that accumulate as a result of such stresses. Increased DELLA activity and stability leads to increased ROS scavenging through the activity of NADPH oxidases[4] which, in turn, results in greater survival. The reduction in ROS induced cell death due to DELLA accumulation may also contribute to an increased resistance to necrotrophic pathogens. This evidence suggests that GA signaling plays a role in coordinating multiple pathways and fits into the emerging view that plant hormones regulate a trade-off between growth and defense mechanisms.

## Emerging role for BR in the regulation of trade-offs between growth and stress tolerance

It has become clear that BR also has a role in modulating plant defense responses. A number of recent studies provide evidence that BR signal induction inhibits basal plant immunity activated by pathogen-associated molecular pattern (PAMP) recognition. It has been shown that PAMP-triggered immunity (PTI) in *Arabidopsis thaliana* is antagonized by activation of the BR signal. Overexpression of BR signaling components in Arabidopsis results in enhanced susceptibility to both bacterial and oomycete pathogens[5]. The brassinosteroid receptor (BRI1) shares a co-receptor (BAK1) with several PAMP receptors, suggesting that competition for BAK1 may be responsible for the observed antagonism. This, however, does not seem to be the case, and the cause of the antagonism appears to lay with BZR1, the key transcription factor regulating BR-responsive genes. BZR1 promotes the expression of a number of WRKY transcription factors that act as negative regulators of ROS production associated with the onset of PTI[5].

BR signalling also interacts with other hormonal signalling pathways leading to impacts on disease resistance. The affect of these interactions on resistance appear to vary with host and pathogen species; however, the general principles have not become clear. In some instances BR appears to act synergistically with SA-mediated resistance while in others the interaction is antagonistic[3]. BR-mediated signalling, similar to that for GA, appears to function in both plant development and disease resistance through a complex integration with other phytohormone signaling pathways. Further investigation into the effects of BR on disease resistance is needed, however, to better understand its role in plant defense and implications for crop improvement.

## Exploiting the *Brachypodium distachyon* pathosystem for model-to-crop translation

Most research into plant hormone signaling has been undertaken using genetic model plant organisms. Arabidopsis is a popular model organism due to its short generation time, sequenced genome (first published in 2000), and variety of genetic resources available. Arabidopsis belongs to the *Magnoliopsida* dicotyledonous class of plants, whilst economically important cereal crops such as wheat (*Triticum* aestivum) and maize (*Zea mays*) belong to the *Liliopsida* class and are monocotyledonous. Differences are known to exist between these classes of plants in terms of phytohormonal regulation, which hinders the transfer of knowledge from this model to cereal crops and suggests that a model more closely related to cereals would be a valuable tool for studies on the role of phytohormones in disease resistance. Rice (*Oryzae sativa*) has been proposed as a monocotyledonous model for cereals such as barley (*Hordeum vulgare*), wheat, and maize. However recent studies suggest that the role of

GA signalling in disease resistance against pathogens of differing trophic styles may not be the same in rice as in the temperate cereals wheat and barley[2,3]. This suggests that it may still be difficult to apply results determined from studies in rice to cereal crops, and that investigation of the potential of other model organisms for this purpose is warranted.

*Brachypodium distachyon* (Bd) is a monocotyledonous plant that has become increasingly prominent as a model for studying plant-pathogen interactions for cereal diseases. The short physical stature, small genome, and rapid life cycle of Bd make it suitable as a reference species for plant research. The availability of a full genome sequence and an increasing number of genetic and genomic resources add to the attractiveness of Bd as a model for monocot studies. Most importantly, Bd has a close phylogenetic relationship with the core of the *Pooideae* subfamily, to which all temperate cereals belong, and also demonstrates a higher gene synteny (the order in which genes are organized within genomes) with cereal crops than other proposed monocot plant systems such as rice. Bd has been shown to serve as a host for an extensive range of pathogen species including *Fusarium graminearum*, *Oculimacula* species, and *Ramularia collo-cygni*[6] that cause agronomically important diseases of wheat and barley. Bd has the potential to act as an ideal model with which to investigate plant-pathogen interactions of relevance to cereals and to study trade-offs. Until recently however, although Bd was shown to act as a host for cereal pathogens, the conservation of function with respect to disease resistance between Bd and temperate cereals had not been demonstrated.

## Attenuation of BR signalling impacts disease resistance in Bd and barley

We recently investigated the conservation of defense mechanisms between Bd and barley, as a proof-of-concept to demonstrate the potential to transfer insight acquired from the model species to cereal crops[7]. Our study also aimed to investigate the impact of BR in broad-range disease resistance against a range of cereal diseases caused by fungal pathogens infecting various host tissues and exhibiting different trophic lifestyles (biotrophs, hemibiotrophs and necrotrophs).

We previously characterized a Bd T-DNA tagged line in which expression of the BR receptor (BRI1) was essentially eliminated. This line exhibits an extremely dwarf phenotype. The barley ‘uzu’ mutant has a mutation in the gene encoding its BR receptor (HvBRI1) and displays a semi-dwarf phenotype. We examined the effect of mutations in these homologous genes in the two species on resistance against *Fusarium culmorum* (Fusarium head blight, root rot, crown rot diseases), *Gaeumannomyces graminis* pv. *tritici* (take-all), *Magnaporthe oryzae* (rice and wheat blast), *Ramularia collo-cygni* (Ramularia leaf spot), *Blumeria graminis* f. sp. *hordei* (powdery mildew), *Oculimacula yallundae*, and *O. acuformis* (eyespot). Disease tests revealed that reduced BR perception enhanced disease resistance against pathogens that exhibit a necrotrophic lifestyle or hemibiotrophs with a short asymptomatic phase (known as the “biotrophic” phase of infection) in both Bd and barley. Resistance induced by BRI1 mutation was observed against infection with *G. graminis* (a true necrotroph), *F. culmorum* (hemibiotroph), *O. acuformis* and *O. yallundae* (necrotroph and hemibiotroph respectively), and *M. oryzae* (hemibiotroph). However, no impact on disease resistance was observed when plants were infected with either biotrophic fungi (*B. graminis*) or hemibiotrophic (*R. collo-cygni*) pathogens exhibiting a prolonged asymptomatic phase. These results indicate that disruption of BR perception has pleiotropic effects on disease resistance and suggest that the BR signal functions antagonistically, with disease resistance effective against necrotrophic and hemibiotrophic pathogens with a short biotrophic phase of infection.

These results provide additional evidence that BR growth-promoting hormone, like GA, influences both plant growth and disease resistance. Semi-dwarf phenotypes conveyed by altered BR or GA signaling are favorable in agriculture, as they prevent lodging under higher nitrogen application resulting in increased crop yield. Barley and wheat *Rht* lines with attenuated GA signaling due to altered DELLA stability or function display increased resistance towards necrotrophic pathogens but increased susceptibility to biotrophic pathogens. This suggests that the GA pathway conveys a resistance trade-off between pathogens of differing trophic lifestyles. Contrastingly, Bd and barley lines with attenuated BR sensitivity exhibit enhanced resistance to necrotrophic pathogens without any discernible increase in susceptibility to biotrophs.

Attenuation of GA signalling also leads to increased abiotic stress tolerance, in part due to enhanced scavenging of ROS by NADPH oxidases. In contrast, we observed that both Bd and barley BR insensitive lines were less tolerant to cold and drought stress. These and previously reported observations suggest that attenuation of BR signalling has the opposite effect to that of attenuated GA signalling on resistance to abiotic stresses[7]. The absence of a disease trade-off in barley lines with altered BR signalling suggests that further manipulation of the BR pathway or BR-regulated genes may be useful when attempting to breed cereal crops with reduced height, without compromising disease resistance. Ultimately, the central role for BR in orchestrating the regulation of growth or defense, as well as modulating the response of plants to resist various types of stresses, whether biotic or abiotic, creates new perspectives in biotechnologies and potential applications for plant breeding. Advances in understanding how the BR and GA pathways interact to regulate responses to a wide array of stresses provides exciting prospects for the development of new crop varieties. Future research should focus on the specific BR-responsive genes responsible for positive and negative impacts on stress tolerance mechanisms in order to develop new cereal varieties containing mutations having positive impacts on biotic and abiotic stress tolerance without detrimental trade-off effects.

In our investigation we explored the use of Bd as a model for temperate cereals with respect to disease resistance. We have demonstrated that it is possible to successfully translate knowledge gained from studies of disease resistance and plant hormone signaling in the model species Bd to cereals such as barley. The results of this proof-of-concept study, coupled with the ease of growth in the laboratory, diversity of natural accessions, and genetic resources, illustrate that Bd is a highly attractive model for investigating resistance mechanisms in economically important cereal diseases. We have identified variation in resistance towards a number of fungal pathogens in natural accessions of Bd (unpublished results). Once the genes responsible for differences in resistance have been identified, it may be possible to exploit synteny to identify potent alleles within cereal species and help to accelerate the development of new varieties and increase our understanding of plant resistance to biotic and abiotic stresses.

**References**


1. Leon-Reyes A, Spoel SH, De Lange ES, Abe H, Kobayashi M, Tsuda S, Millenaar FF, Welschen RAM, Ritsema T, Pieterse CMJ (2009). Ethylene modulates the role of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 in cross talk between salicylate and jasmonate signaling. Plant Physiology. 149:1797–809.
2. Saville RJ, Gosman N, Burt CJ, Makepeace J, Steed A, Corbitt M, Chandler E, Brown JKM, Boulton MI, Nicholson P (2012). The ‘Green Revolution’ dwarfing genes play a role in disease resistance in *Triticum aestivum* and *Hordeum vulgare*. Journal of Experimental Botany. 63:1271-1283.
3. De Bruyne L, Höfte M, De Vleeschauwer D (2014). Connecting growth and defense: The emerging roles of brassinosteroids and gibberellins in plant innate immunity. Molecular Plant. 7:943-959.
4. Lozano-Duran R, Zipfel C (2014). Trade-off between growth and immunity: role of brassinosteroids. Trends in Plant Sciences. Doi:10.1016/j.tplants.2014.09.003.
5. Achard, P and Genschik, P. (2009). Releasing the brakes of plant growth: how GAs shutdown DELLA proteins. Journal of Experimental Botany. 60: 1085-1092.
6. Peraldi A, Griffe LL, Burt C, McGrann GRD, Nicholson P (2014). *Brachypodium distachyon* exhibits compatible interactions with *Oculimacula* spp. and *Ranularia collo-cygni*, providing the first pathosystem model to study eyespot and Ramularia leaf spot diseases. Plant Pathology. 63: 554-562.
7. Goddard R, Peraldi A, Ridout C, Nicholson P (2014). Enhanced disease resistance caused by BRI1 mutation is conserved between Brachypodium distachyon and barley (Hordeum vulgare). MPMI. 27:1095-1106.


*Antoine Peraldi, Rachel Goddard and Paul Nicholson*
*John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK*
*antoineperaldi@googlemail.com or rachel.goddard@jic.ac.uk*

# Heterogeneous Consumer Preferences for Nanotechnology and Genetic-Modification Technology in Food Products

*Chengyan Yue, Shuoli Zhao and Jennifer Kuzma*

The application of novel technologies to food such as biotechnology and nanotechnology continues to grow rapidly. Food with genetically engineered ingredients currently constitutes a large portion of domestic food supply in the U.S., including an estimated 70% of processed foods[1]. While the use of nanotechnology is presently limited in the food market, the prospect for its growth in the food supply and economy is significant. Numerous companies are pursuing food nanotechnology applications for release to both domestic and international consumer markets in the near and long term, with an estimated rise in total market value to $20 billion in 2015[2]. Given the strong prevalence and interest in food produced using genetic modification (GM food) and nanotechnology (nano-food), it is important to understand consumer perceptions of the benefits and risks, as well as to understand how product acceptance is affected by price, labeling, and these risks and benefits.

## Public Perceptions of GM Food and Nano-food

Potential benefits and risks of both technologies have been raised in the academic literature and debated in the media. For GM food, despite the anticipated benefits such as pesticide reduction, taste enhancement, and shelf-life extension[3], the application of GM technology in food has generated widespread public controversy. Many studies investigating consumer preference for GM foods indicate that GM foods are generally not favored by consumers. Some of the reasons behind this opposition include people's previous knowledge of GM technology, risk perception of and safety concerns about the technology, distrust in government management of the technology and media coverage of the potential risks, and cultural world views[4]. In contrast, compared to consumers from developed countries, consumers from less developed countries tend to have more positive attitudes toward GM foods, possibly due to perceived benefits to food security and increased production[5]. Regardless, across studies and countries, consumers are generally willing to pay a premium for foods free of GM ingredients (of about 10% to 50%) while the magnitude of consumers' discount for GM foods depends upon the type of genetic modification, the type of food product, and how the genetic modification alters the final product.

Nanotechnology applications to food products, though less known by the public, are also expected to bring a range of benefits, including improved taste, less fat, enhanced absorption of nutrients, improved food safety, and traceability[6]. In past nanotechnology and public perception research, nano-food products were not the focal point. However, a few existing studies taken place in recent years indicated that consumers prefer natural additives over nanomaterial additives with added benefits[7]. Health information about nanotechnology significantly decreases consumers' willingness to pay (WTP) for nano-food, while societal and environmental information did not have significant impacts[8]. Also, people tend to prefer applications of nano-food that fill needs of nutrition or safety, and they are more comfortable with nanomaterials in packaging than within the food itself[9].

Although GM and nano-foods are notably similar as applications of novel broad-based technologies to food in uncertain public knowledge contexts, some notable differences exist. GM foods involve primarily "genetic" changes to ingredients whereas nano-food applications usually apply "chemical" or structural changes. GM foods are already prevalent on the market, while nano-foods are just emerging. GM foods have had high profile media and policy debates (e.g., California's recent labeling proposition), whereas nano-foods have not. Consumers' conceptualization of nanotechnology in food may be more nuanced or differently developed than their conceptualization of GM food. Given such mixture of similarities, contrasts, and differing market prevalence, we carried out direct comparison of the two technologies and of associated benefits, and further explored heterogeneity in consumer preference to better understand the range of consumer differences under a reasonable segmentation of latent groups.

## Experimental Design and Process

In this study, choice experiments were conducted to elicit consumer preference and WTP for the technology and benefit attributes of rice. Choice experiments are widely used by researchers to investigate consumer preferences and WTP for goods. They are based on random utility theory and Lancaster's consumer demand theory[10], implying that consumers derive utility from attributes of a good rather than from the good itself. Choice experiments are composed of a combination of attributes, so they allow researchers to value various attributes simultaneously. We chose rice for this experiment because it is a product most people consume in a year, does not generally present food allergens, is modestly priced, and has been proposed for nutritional enhancement through genetic engineering (e.g., Golden Rice).

During the experiment, participants were presented with a series of choice scenarios of 32oz (2lb) bag of long grain white rice, which consisted of varied combinations of the product attributes listed in **Table 1**. To reduce the cognitive burden on participants in the choice experiment, only two alternatives were included in each scenario along with an opt-out option indicating that neither of the two alternatives is preferred. We also provided general information to familiarize participants with the two technologies before they made their choices. Choice experiment data were collected in August 2013 using an online survey by QualtricsTM, a professional survey company. A total of 1117 complete online surveys were received, and the socio-demographic characteristics of our sample are consistent with the U.S. census (U.S. census, 2010) in terms of age (age group 15-83), gender, family income, and education.

Latent class model assumes that individuals can be intrinsically sorted into a number of latent classes, that each class is characterized by homogenous preferences, and that the preferences are heterogeneous across classes. Therefore, it not only allows continuous heterogeneity, but also well suited for explaining sources of heterogeneity

Based on the coefficients and attribute/price ratios (WTP values) of technologies and benefits for each latent class defined, we named the four estimated latent classes as "Price Oriented/Technology Adopters," "Technology Averse," "Benefit Oriented," and "Technology Rejecters," respectively. Each group's WTP values for the combinations the technologies and attributes are reported in **Table 2**.

Group 1 is the "Price Oriented/Technology Adopters" group, which accounts for 17.64% of the sample. In this group, the absolute value of the coefficient for price is the highest among all parameters, which means participants in group 1 are the most sensitive to the change in price. The Price Oriented/Technology Adopters group discounts GM slightly more than nanotechnology. Further for this group, the enhanced nutrition is the most preferred benefit followed by improved taste and safety. The coefficient for less harmful impact on environment is not significant. According to the WTP values, participants in this group are willing to pay the highest premium for nutrition enhancement with a premium $0.33/lb for nanotechnology, and a premium of $0.32/lb for GM. In contrast, this group is not willing to pay for either of the technologies to reduce harmful impact on the environment. Since the combined WTP estimates of group 1 are either slightly positive (but statistically significant) or statistically insignificant, we also call this group of participants the "Technology Adopters."

Group 2, the "Technology Averse" group, consists of 192 participants (17.2% of the sample). The most obvious feature for this group is that the coefficients for nanotechnology and GM are significantly negative, whereas the coefficients for benefit attributes are comparatively less positively significant. In this group, participants are strongly against the use of GM and nanotechnology, and the negative coefficients for the two technologies dominate the positive coefficients of the four benefits. Overall, Technology Averse participants discount the combinations of GM and benefits more than the combinations of nanotechnology and benefits. For the GM application in food products, Technology Averse participants discount taste improvement the most, followed by less harmful impact on the environment, nutrition enhancement, and safety improvement. The same preference ranking applies to the WTP results for benefits brought by nanotechnology.

Group 3, the "Benefit Oriented" group, is the majority segment that consists of 40.3% of total participants. This group is called "Benefit Oriented" as the coefficients of the benefit attributes are significantly positive and they dominate the negative coefficients of the technologies. This indicates that participants in this group do not care about what technology is adopted during production as long as certain benefits can be brought by the technology. Within the four benefits, this group values safety the most, followed by nutrition enhancement, less harmful impact on the environment ranks the third, and improved taste is the least preferred benefit. As a result, all the WTP values for the combinations of the technologies and the benefits are significantly positive at 0.001 level. The results indicate about 40% of participants prefer the use of nanotechnology and GM to improve benefits for food products. Using nanotechnology to improve food safety is preferred the most ($5.03/lb), and employing GM to improve taste is preferred the least ($1.93/lb).

Group 4, "Technology Rejecters," has 278 participants (24.9 % of the total sample). Based on the estimation results of the latent class logit model, the coefficients of nanotechnology and GM strongly dominate the coefficients of price and benefits. Participants in this group reject to choose nanotechnology or GM regardless of the associated benefits and prices whenever there is a conventional option. Technology Rejecters discount GM and nanotechnology by $-3.39/lb and $-3.90/lb, respectively, and the highest price premium they are willing to pay is $1.10/lb for improved food safety. All technology and benefit combinations are significantly discounted from -$3.53/lb (GM with less harmful impact to environment) to -$2.29/lb (nanotechnology with enhanced food safety).

## Characteristics across four latent groups

The mean statistics of socio-demographics and religious

**Table 1. Choice Experiment Attribute and the Corresponding Attribute Levels**

| Attribute | Level |
|---|---|
| The **Production Technology** used to produce the rice | Nanotechnology<br>Genetic Modification<br>Conventional |
| The type of **Benefit** that could be attained by using the given technology | Enhanced nutrition<br>Improved product taste<br>Improved food safety of the rice<br>Less harmful impact on the environment during production<br>No additional benefit |
| Product **Price** for a 32 oz (2 lb) bag of long grain white rice | $3.75<br>$5.00 |

**Table 2. WTP for the Combinations of Technologies and Benefits for the Four Consumer Segments**

| WTP($/lb) (95% CI) | Group 1: Price Oriented | | Group 2: Technology Averse | | Group 3: Benefit Oriented | | Group 4: Technology Rejecters | |
|---|---|---|---|---|---|---|---|---|
| | Nano-technology | GM | Nano-technology | GM | Nano-technology | GM | Nano-technology | GM |
| Nutrition | 0.33***<br>(0.25,0.40) | 0.32***<br>(0.24,0.39) | -0.49***<br>(-0.65,-0.34) | -0.57***<br>(-0.73,-0.41) | 4.22***<br>(2.37,6.08) | 4.10***<br>(2.27,5.94) | -2.83***<br>(-3.59,-2.06) | -3.33***<br>(-0.17,-2.50) |
| Safety | 0.12**<br>(0.04,0.21) | 0.11**<br>(0.03,0.20) | -0.31***<br>(-0.46,-0.16) | -0.38***<br>(-0.53,-0.24) | 5.03***<br>(2.91,7.15) | 4.91***<br>(2.82,6.99) | -2.29***<br>(-2.93,-1.64) | -2.79***<br>(-3.54,-2.05) |
| Environment | -[a] | - | -0.60***<br>(-0.77,-0.42) | -0.67***<br>(-0.85,-0.49) | 3.15***<br>(1.78,4.52) | 3.03***<br>(1.68,4.38) | -3.02***<br>(-3.82,-2.23) | -3.53***<br>(-442,-2.64) |
| Taste | 0.23***<br>(0.17,0.30) | 0.22***<br>(0.15,0.30) | -0.70***<br>(-0.88,-0.53) | -0.78***<br>(-0.96,-0.60) | 2.05***<br>(1.07,3.03) | 1.93***<br>(0.97,2.89) | -2.83***<br>(-3.57,-2.10) | -3.34***<br>(-4.14,-2.54) |

Notes: a: "-" indicates that the estimated coefficient is not statistically significant, and the WTP value is not calculated. b: a single asterisk (*), double asterisks (**), and triple asterisks (***) denote significance at the 0.05, 0.01 and 0.001 levels, respectively.

attitudes differ significantly across groups, and **Figure 1** summarizes the major characteristics of the four latent groups.

"New Technology Rejecters" are the oldest with an average age of 50, and "Benefit Oriented" consumers are the youngest with the average age of 45, which is consistent with the level of conservative tendencies. This is in line with previous findings that older participants are more likely to know about and value traditional food[11]. The "Technology Averse" group has the lowest income level while participants in the other three groups have relatively similar income levels. Participants in "Benefit Oriented" and "Technology Averse" groups have relatively lower education levels while participants in the other two groups have higher education levels. "New Technology Rejecters" and "Technology Adverse" groups have significantly more female members, which suggests that the technologies are less acceptable to female participants compared to male participants.

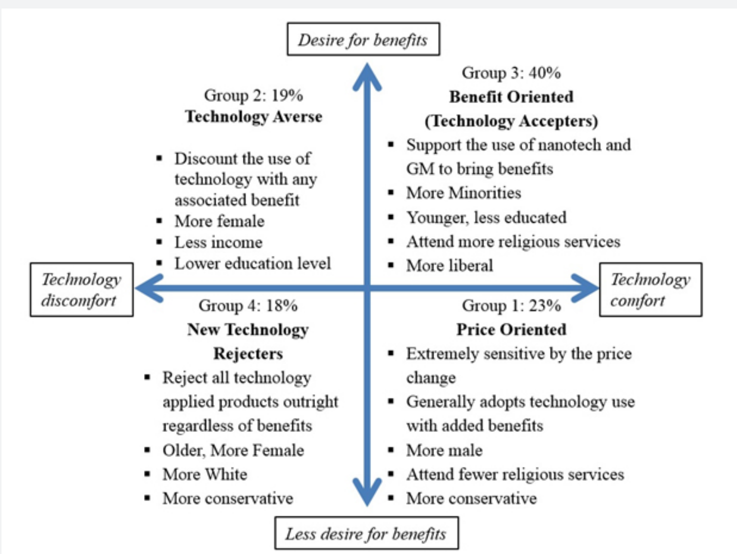


**Figure 1. Characteristics of the four consumer segments**

In looking at the political views of the consumers, it is interesting to note the dichotomy between the two meta-groups identifying as more liberal (Benefits Oriented) and those as more conservative (the other three groups). Perhaps this could be explained by different factions of conservative people rejecting the technology outright for moral reasons (consistent with the Rejecters), or alternatively accepting the technology for economic development and free-market reasons (consistent with the Price Orientation).

Finally, religious background may not necessarily affect their choice of food products produced using certain production technologies, as the two variables of how religion affects views of science and technology, and decisions about them were not significant. This is consistent with a previous finding that religiosity does not affect people's attitudes toward nanotechnology[12].

## Possible Policy Implication

From a policy perspective, our results suggest that the majority of U.S. consumers will not reject these technologies outright, but base their decisions on a complex calculus of benefits, risks, technological comfort, and safety. However, we do find a group of people (approximately 25% of the population) who oppose the application of nanotechnology or GM in the production of rice regardless of price level and corresponding benefits. This is consistent with a growing social movement in the U.S. towards "natural foods."

Wise policy choices and product development strategies might allow the New Technology Rejecters group (Group 4) to make their own decisions by labeling nano-foods or GM foods, either through voluntary industry initiatives or mandatory government initiatives, while making sure that nano-foods and GM foods provide benefits in safety and nutrition through industry product developments or government policy incentives to satisfy the desires of other groups (Group 3). For the food industry, claims of increasing food quality and safety through technology should be verified for increasing acceptance in the majority of consumers who are somewhat open to technological advances (Groups 2 and 3), while keeping price reasonable for those who are eager to adopt technologies (Group 1) and allowing those who reject technology to choose (Group 4).

**Source: Yue, C., Zhao, S. and Kuzma, J. (2014), Heterogeneous Consumer Preferences for Nanotechnology and Genetic-modification Technology in Food Products. Journal of Agricultural Economics. doi: 10.1111/1477-9552.12090**

**References**

1. Hallman, W. K., Hebden, W. C., Aquino, H. L., Cuite, C. L. and Lang. J. T. Public Perceptions of Genetically Modified Food: A National Study of American Knowledge and Opinion. Food Policy Institute Publication, 2003. No. RR-1003-004, Rutgers University, New Brunswick, NJ.
2. Groves, K. and Titoria. P. 'Nanotechnology and the food industry', *Food Science and Technology*, Vol. 23(2), (2009) pp. 18-20.
3. Rojas-Méndez, J. I., Ahmed, S. A., Claro-Riethmüller, R. and Spiller, A. 'Acceptance of genetically modified foods with health benefits: a study in Germany', *Journal of Food Products Marketing*, Vol. 18(3), (2012) pp. 200-221.
4. Lusk, J. L., Jamal, M., Kurlander, L., Roucan, M., & Taulman, L. 'A Meta-Analysis of Genetically Modified Food Valuation Studies.' *Journal of Agricultural and Resource Economics*, 30(1), (2005) pp. 28-44.
5. Curtis, K. R., McCluskey, J. J. and Wahl, T. I. 'Consumer acceptance of genetically modified food products in the developing world', AgBioForum, 7(1&2), (2004) 70–75.
6. Chaudhry, Q., Watkins, R. and Castle. L. 'Nanotechnologies in the food arena: new opportunities, new questions, new concerns', *The Royal Society of Chemistry*, (2010) pp. 1-17.
7. Siegrist, M., Cousin, M. E., Kastenholz, H. and Wiek, A. 'Public acceptance of nanotechnology foods and food packaging: the influence of affect and trust', *Appetite*, Vol. 49, (2007) pp.459–466.
8. Marette, S., Roosen, J., Bieberstein, A., Blanchemanche, S. and Vandermoere, F. 'Impact of environmental, societal and health information on consumers' choices for nanofood', Journal of Agricultural & Food Industrial Organization, Vol. 7(2), (2009) a Impact of environmental, societal and health information on consumers' choices for nanofood rticle 11.
9. Brown, J. and Kuzma, J. 'Hungry for information: public attitudes toward food nanotechnology and labeling', Review of Policy Research, Vol. 30, (2013) pp. 512–548.
10. Lancaster, K. J. 'A new approach to consumer theory', The Journal of Political Economy, Vol. 74(2), (1966) pp. 132–157.
11. Stewart, P. 'Consumption choices concerning the genetically engineered, organically grown, and traditionally grown foods: an experiment', *Knowledge, Technology and Policy*, Vol. 13(1), (2000) pp. 58-69.
12. Hossain, F., and Onyango, A. 'Product attributes and consumer acceptance of nutritionally enhanced genetically modified foods', *International Journal of Consumer Studies* Vol. 27(3), (2004) pp, 255-267.

*Chengyan Yue*
*Bachman Endowed Chair in Horticultural Marketing*
*Associate Professor*
*Departments of Applied Economics and Horticultural Science*
*University of Minnesota – Twin Cities*
*yuechy@umn.edu*

*Shuoli Zhao*
*Departments of Applied Economics*
*University of Minnesota – Twin Cities*
*zhao0468@umn.edu*