MON 87460 Corn Safety Summary[1]

Summary Overview

The adoption of MON 87460 corn by growers is expected to provide an additional tool to manage risks associated with drought stress. While much progress has been made to improve corn yield in water-limited environments through breeding, selection, and agronomic management practices, there remains potential for additional improvement. Higher corn yield per acre may help mitigate the utilization of additional acres to meet the future needs for food, feed, and biofuel uses or produce more corn grain on the same number of acres already used for corn production. Positive impacts on yield and improved yield stability will provide value to producers, consumers, and the environment. The data and information presented below were collected to demonstrate the familiarity of MON 87460 corn as compared to conventional corn and, moreover, show that MON 87460 corn has no greater weediness or plant pest potential.

Product Description

Corn is a versatile crop that provides food, feed, and fuel to a global economy. Recently, a surge in demand for corn has been created by growing economies in the developing world and its use as an alternative fuel source in the developed world. These demands may exceed production and lead to diminished grain reserves. In addition, climate change may have variable impacts on crop yields, potentially creating further supply disruptions. The combination of these factors places a premium on corn yield stability in sub-optimal environments.

Corn is the largest crop grown in the United States in terms of acreage planted and net value. Drought stress is typically the major cause of yield reduction in corn and its effects have far reaching global economic implications. In North America alone, it is estimated that 40% of annual crop losses are due to sub-optimal water availability. Consequently, increasing drought tolerance in corn is a major goal of breeding selection and biotechnology. Advances in conventional plant breeding and agronomic practices have made contributions to increasing corn drought tolerance and yield potential. Nearly all corn hybrids currently on the market have been bred to exhibit some degree of drought tolerance. Biotechnology provides a significant additional tool that can be used in combination with conventional breeding and agronomic practices to increase corn yield stability under water-limited conditions.

MON 87460 corn, developed by the use of recombinant DNA techniques, reduces yield loss under water-limited conditions compared to conventional corn. Under well-watered conditions, grain yield for MON 87460 corn is equivalent to conventional corn. Like conventional corn, MON 87460 corn is still subject to yield loss under water-limited conditions at key development stages, particularly during flowering and grain fill periods when corn yield potential is most sensitive to stress by disrupting kernel development. Under severe water deficit, corn grain yield for MON 87460 corn, as with other corn hybrids, can be reduced to zero.

MON 87460 corn expresses a cold shock protein B (CSPB) produced from the inserted *Bacillus subtilis*-derived gene, and neomycin phosphotransferase II (NPTII) that was used as a selectable

[1] For detailed information see USDA Petition for the Determination of Non-regulated Status of MON 87460

marker during transformation. In bacteria, the CSPB protein helps preserve normal cellular functions during certain stresses by binding cellular RNA and unfolding secondary structures that could affect RNA stability and translation. As in bacteria, the CSPB protein in MON 87460 corn binds with corn RNA helping to maintain plant cellular functions under drought stress. Data suggest that MON 87460 corn reduces yield loss, primarily through increased kernel number per ear, under water-limited conditions by minimizing the effect of water limitation on photosynthesis, stomatal conductance, and carbon fixation. On a plant level, corn yield losses associated with drought stress occur as a result of reduced synchrony between anthesis and silking, embryo loss, and/or reduced grain filling in viable kernels. Studies on corn hybrids with enhanced drought tolerance developed using conventional breeding show that yield improvements are attained through improvements in all of these same endpoints. Therefore, the enhanced yield stability of MON 87460 corn under water-limited conditions, conferred by the expressed CSPB protein, appears to be the result of improvements in the natural stress response mechanisms in conventional corn.

Water Management Regimes Employed for Field Trials Designed Specifically for this Product

To demonstrate MON 87460 corn reduces yield loss under water-limited conditions, field trials were designed to assess MON 87460 corn across a broad range of soil moisture and environmental conditions relevant to where commercial production is expected. MON 87460 corn was compared to a conventional control, which had a genetic background similar to MON 87460 corn but did not possess the drought tolerance trait. In addition, multiple conventional corn hybrids (references) that possess a range of drought tolerance imparted through conventional breeding were included in the analysis to establish a range of natural variability for each measured characteristic. The commercial reference hybrids selected were adapted to the geographic region relevant to each field site; as such, each reference hybrid included some degree of drought tolerance conferred through conventional breeding.

Field trials were established using three different water management regimes: (1) well-watered, (2) both well-watered and water-limited conditions established in the same field, or (3) water managed according to typical or standard agronomic practices, which included typical amounts of supplemental irrigation at relevant sites. Fields established under well-watered conditions allowed an evaluation of MON 87460 corn in the absence of trait bias, where no statistical differences in yield were expected between MON 87460 corn and the control. Fields managed for both well-watered and water-limited conditions allowed an evaluation of MON 87460 corn under limited soil moisture conditions where it is expected to result in reduced yield loss. Finally, fields managed according to typical agronomic practices allowed an evaluation of MON 87460 corn under a natural range of environmental conditions relevant to commercial corn production regions. Data and plant tissue samples generated from these fields with various water management regimes were used to assess the safety of MON 87460 corn under different soil moisture conditions including: (1) expression levels of the introduced proteins in several tissue types, (2) compositional analysis of forage and grain, (3) phenotypic and agronomic characteristics at several plant development stages, and (4) environmental interactions. The variation in yield response observed among MON 87460 corn, the control, and commercial reference hybrids in these fields is within normal levels expected for conventional corn. Therefore, the data from these field trials are relevant to the assessment of MON 87460 corn under different water management regimes. In the water-limited sites, the data are relevant to

demonstrate the efficacy of MON 87460 corn. MON 87460 corn is expected to provide reduced yield loss under most water-limited conditions compared to conventional corn

Data and Information Confirm the Food and Feed Safety and no Adverse Effect on the Environment for MON 87460 Corn Compared to Conventional Corn

All available data and information demonstrate the safety of MON 87460 corn as compared to conventional corn and, moreover, show that MON 87460 corn will not have an adverse effect on the environment. The food, feed, and environmental safety of MON 87460 corn was confirmed based on multiple, well-established lines of evidence:

1. Corn is a familiar crop that does not possess any of the attributes commonly associated with weeds, has a history of safe consumption, and serves as an appropriate basis of comparison to support the safety assessment of MON 87460 corn.
2. A molecular characterization of the inserted DNA in MON 87460 corn confirmed the insertion of a single functional copy of the *cspB* and neomycin phosphotransferase II (*nptII*) expression cassettes at a single locus within the corn genome.
3. A detailed biochemical characterization was conducted on the CSPB and NPTII proteins produced in MON 87460 corn. Both proteins are readily digested and have long histories of safe use and are expressed at low levels in MON 87460 corn.
4. An assessment of the allergenicity and toxicity potential of the CSPB and NPTII proteins demonstrated that the CSPB and NPTII proteins are unlikely to be allergens, toxins, or similar to other biologically active proteins known to have adverse effects on mammals.
5. A compositional and nutritional assessment confirmed that MON 87460 corn forage and grain are compositionally equivalent to conventional corn.
6. An extensive evaluation of phenotypic and agronomic characteristics and environmental interactions demonstrated that MON 87460 corn poses no increased plant pest potential, including weediness potential, and no adverse environmental impact compared to conventional corn.
7. An assessment on the potential impact to non-target organisms (NTO) demonstrated that MON 87460 corn is unlikely to have an adverse effect on these organisms.
8. The introduction of MON 87460 corn is no more likely to have an impact on land use, cultivation practices, or the management of weeds, diseases, and insects than the use of conventionally bred drought tolerant corn.

These lines of evidence are described in further detail in the following sections.

Corn is a Familiar Crop Lacking Weedy Characteristics

Corn is grown extensively throughout the world, and is the largest cultivated crop followed by wheat (*Triticum* sp.) and rice (*Oryza sativa* L.) in total global production. In the U.S., corn is grown in almost all states and is the largest crop grown in terms of acreage planted and net value. Corn has been studied extensively, and the domestication of corn can be traced back to approximately 10,000 years ago in southern Mexico.

Corn is not listed as a weed in the major literature references, nor is it considered to be a noxious weed species by the federal government. In addition, corn has been grown throughout the world without any report that it is a serious weed. Corn is poorly suited to survive in the environment without human assistance and is not capable of surviving as a weed due to past breeding selection in the domestication of corn. During domestication of corn, innate traits often associated with weediness, such as seed dormancy, a dispersal mechanism, or the ability to establish reproducing populations outside of cultivation, have not been selected. Similarly, there is no indication from the history of hybrid corn breeding in the U.S. of changes that could alter the weediness profile of the crop. Although corn seed can overwinter into a rotation with soybeans or other successive crops, mechanical and chemical measures are routinely used to control corn volunteers. Some populations of wild annual and perennial species that could hybridize with MON 87460 corn are known to exist in the U.S., however key differences in several factors such as flowering time, geographical separation, and development timings make natural crosses in the U.S. highly unlikely.

A conventional corn comparator was used to support the safety assessment of MON 87460 corn. The conventional corn comparator had a similar genetic background as MON 87460 corn without the inserted *cspB* and *nptII* expression cassettes. Comparisons of MON 87460 corn with the conventional corn comparator allowed the effect of the *cspB* and the *nptII* expression cassettes and the expressed CSPB and NPTII proteins to be assessed in an unbiased manner.

Molecular Characterization Verifies the Integrity and Stability of the Inserted DNA in MON 87460 Corn

MON 87460 corn was produced by *Agrobacterium*-mediated transformation of corn with PV-ZMAP595, a binary vector containing a single transfer DNA (T-DNA). The T-DNA contains two expression cassettes. The first expression cassette encodes the CSPB protein and the second expression cassette encodes for the NPTII protein, a selectable marker that was used during product development. During transformation, the T-DNA was inserted into the genome. Molecular characterization of MON 87460 corn by Southern blot analyses demonstrated that the DNA inserted into the corn genome is present at a single locus and contains one functional copy of the *cspB* and the *nptII* expression cassettes. All genetic elements are present in the inserted DNA as expected. No backbone plasmid DNA sequences outside of the T-DNA were detected. PCR and DNA sequence analyses provided the complete DNA sequence of the insert and confirmed the organization of the elements within the insert.

The stability of the integrated DNA is demonstrated by the fact that the Southern blot fingerprint of MON 87460 corn was maintained for multiple generations tested in the breeding history. The stability was further confirmed by the fact that the inheritance of the T-DNA in MON 87460 corn follows Mendelian patterns of segregation.

Data Confirm CSPB and NPTII Protein Safety

A multistep approach was used to characterize and assess the safety of the CSPB and NPTII proteins expressed in MON 87460 corn. This detailed characterization confirms the CSPB and NPTII proteins are safe for human and animal consumption. The assessment involved: 1) characterizing the physicochemical and functional properties of the proteins; 2) quantifying protein levels in MON 87460 corn plant tissues; 3) examining the similarity of the CSPB and NPTII proteins to known allergens, toxins and other biologically-active proteins known to have adverse effects on mammals; 4) evaluating the digestibility of CSPB and NPTII proteins in simulated gastrointestinal fluids; 5) documenting the history of safe consumption of CSPB and NPTII proteins; and 6) investigating potential mammalian toxicity through oral ingestion (gavage) evaluations. Results confirm that CSPB is expressed in all MON 87460 corn tissues collected, including stover, root, forage, grain, leaf, pollen, and silk. NPTII is expressed in leaf, root, and forage, and below the limit of detection in grain. CSPB and NPTII have no relevant amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which can have adverse effects on mammals. Studies utilizing the CSPB and NPTII proteins demonstrated that these proteins degraded rapidly in simulated gastric and intestinal fluids and mouse acute oral toxicity evaluations demonstrated the proteins are not acutely toxic and do not cause adverse effects at the highest doses tested. The safety assessment supports the conclusion that dietary exposure to CSPB and NPTII proteins derived from MON 87460 corn poses no meaningful risk to human or animal health.

MON 87460 Corn is Compositionally Equivalent to Conventional Corn

A detailed compositional assessment of MON 87460 corn confirmed that it is as safe and nutritious as conventional corn. The composition of MON 87460 corn was determined from forage and grain tissues produced during two growing seasons from multiple field sites in the major corn production regions of the U.S. and Chile. In each assessment, MON 87460 corn was compared to an appropriate conventional control, which had a genetic background similar to MON 87460 corn but did not possess the inserted *cspB* and *nptII* expression cassettes. In addition, multiple conventional corn hybrids (references) that possess a range of drought tolerance imparted through conventional breeding were included in the analysis to establish a range of natural variability for each compositional component, where the range of variability is defined by a 99% tolerance interval for that particular component. Compositional analyses of the forage samples included proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals (calcium, phosphorous), and carbohydrates by calculation. Compositional analyses of the grain samples included proximates (protein, fat, ash, and moisture), ADF, NDF, total dietary fiber (TDF), amino acids, fatty acids (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium, and zinc), vitamins (B_1, B_2, B_6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic acid, and p-coumaric acid), and carbohydrates by calculation. In all, 77 components were evaluated as part of the nutritional assessment of MON 87460 corn: nine in forage and 68 in grain.

In the U.S. during 2006, six sites were established using typical water management practices as a standard assessment under a natural range of environmental conditions. In Chile during 2006/2007, the experimental design included well-watered and water-limited treatments to

evaluate the effect of soil moisture level on component levels. Across both years of data there were no consistent differences in composition between MON 87460 corn and the control.

In the analysis of samples from U.S. trials, 62 components were statistically assessed as values for 15 components were below the analytical limit of quantitation, and there were 434 comparisons conducted on MON 87460 corn based on seven sets of analyses of data from each of the six field sites plus combination data of all six field sites. In the combined-site analysis there were no significant differences ($p>0.05$) between MON 87460 corn and the conventional control for 59 (95.2%) of 62 comparisons. Furthermore, results from the U.S. 2006 study show that there were no significant differences ($p>0.05$) for 407 (93.7%) of the 434 total comparisons made between the MON 87460 corn and the control. The 27 detected differences were not consistent across sites, were small in magnitude and the mean component values of MON 87460 corn and the control were within the 99% tolerance interval established from the commercial reference hybrids that were produced at the same time and field sites as MON 87460 corn and the control.

In the analysis of samples from trials in Chile, 61 components were statistically assessed as values for 16 components were below the analytical limit of quantitation, and there were 244 comparisons conducted on MON 87460 corn based on four sets of analyses of data from each of the three field sites plus combination data of all three field sites. In the combined-site analysis of the well-watered plots, there were no significant differences ($p>0.05$) between MON 87460 corn and the conventional control for 59 (96.7%) of 61 comparisons. Furthermore, results from well-watered plots show that there were no significant differences ($p>0.05$) for 230 (94.3%) of the 244 total comparisons made between MON 87460 corn and the control. The detected differences were not consistent across sites, were small in magnitude, and the mean component values of the test and control substances were within the 99% tolerance interval established from the commercial reference hybrids that were produced at the same time and field sites as MON 87460 corn and the control. In the combined-site analysis of the water-limited plots, there were no significant differences ($p>0.05$) for 59 (96.7%) of 61 comparisons. Furthermore, results from the water-limited plots show that there were no significant differences ($p>0.05$) for 233 (95.5%) of the 244 total comparisons made between MON 87460 corn and the control. The detected differences were not consistent across sites, were small in magnitude, and the mean component values of the test and control substances were within the 99% tolerance interval established from the commercial reference hybrids. Grain and forage samples from the Chile study were also analyzed for 11 secondary metabolites that are potentially associated with drought stress. Results from this additional analysis further confirm that MON 87460 corn is compositionally equivalent to conventional corn. All compositional analyses, therefore, support the conclusion that MON 87460 corn is not different from conventional corn when grown under a broad range of environmental conditions.

MON 87460 Corn Does Not Change Weediness, Plant Pest Potential or Environmental Interactions Compared to Conventional Corn

Plant pest potential of a biotechnology-derived crop is assessed from the basis of familiarity that the USDA recognizes as an important underlying concept in risk assessment. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a conventional plant hybrid or variety whose biological properties, weediness and plant pest potential are well known. Familiarity considers the biology of the plant, the introduced trait, the

receiving environment, and the interactions among these factors. This provides a basis for comparative risk assessment between a biotechnology-derived plant and a conventional plant hybrid or variety as the control. Thus, the phenotypic, agronomic, and environmental interaction assessment of MON 87460 corn included a genetically similar conventional control as a comparator. This evaluation used a weight-of-evidence approach and considered statistical differences between MON 87460 corn and the conventional control with respect to reproducibility, magnitude, and directionality (trends). Characteristics assessed included: seed dormancy and germination, pollen morphology, and plant phenotypic observations and environmental interaction evaluations conducted in the field. Certain characteristics can be used to assess weediness potential, including seed germination and dormancy (hard seed), pre-harvest seed loss characteristics (lodging and ear drop), and the potential to volunteer in cultivated areas or survive outside cultivation.

The field evaluation of phenotypic, agronomic, and environmental characteristics supports the conclusion that MON 87460 corn is not likely to have increased weediness or plant pest potential compared to conventional corn. Field trials were conducted in the major corn production regions of the U.S. and Chile during 2006 and 2007, and are summarized in the table below. Six field trials totaling 31 sites were established using the three water management regimes described above: 1) well-watered (17 sites); 2) well-watered and water-limited treatments (9 sites); and 3) water managed according to typical local agronomic practices and water conditions (5 sites). The evaluation of 14 phenotypic and agronomic characteristics was conducted in each field study, which included seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped ears, stalk lodging, root lodging, final stand count, grain moisture, test weight, and grain yield.

MON 87460 Field Trials conducted in the U.S. and Chile during 2006 and 2007

Water Management Practice	Country and Year	No. Sites
Well-watered	U.S. 2006	8
Well-watered	U.S. 2007	9
Well-watered & Water-limited	Chile 2006/2007	4
Well-watered & Water-limited	U.S. 2007 (Study-1)	2
Well-watered & Water-limited	U.S. 2007 (Study-2)	3
Typical agronomic practice	U.S. 2006	5

Results from the combined-site analyses across all the six distinct trials detected only four instances of a statistically significant ($p>0.05$) phenotypic difference between MON 87460 corn and the control. For the well-watered regime, two separate trials totaling 17 sites were established in the U.S. during 2006 (8 sites) and 2007 (9 sites). In the combined-site analyses of these data no differences were detected between MON 87460 corn and the control in the 2007 study. In the 2006 study, an increase in root lodged plants was detected for MON 87460 corn compared to the control. For the well-watered and water-limited regime, three different studies totaling nine field sites were established in Chile (4 sites in 2006/2007) and the U.S. (5 sites in

total from two separate trials in 2007). In the Chile 2006/2007 study, no phenotypic differences were detected with the exception of the expected increase in yield for MON 87460 corn compared to the control under water-limited conditions. In the U.S. 2007 Study-1, no phenotypic differences were detected in either the well-watered or water-limited treatments. In the U.S. 2007 Study-2, stay green rating (more green tissue) was lower for MON 87460 corn compared to the control in both the well-watered and water-limited treatments, respectively. Under the typical agronomic practices regime, one study with five sites was established in the U.S. during 2006 and no differences between MON 87460 corn and the control were detected in this study. The scale of these trials was small, though, and not intended to assess small but meaningful differences in yield.

Under a range of temperature regimes, no biologically meaningful differences were detected in the germination and dormancy of seed from MON 87460 corn. In particular, the absence of hard seed supports a conclusion of no increased weediness potential of MON 87460 corn compared to conventional corn for germination and dormancy characteristics. No differences were detected in pollen morphology or viability between MON 87460 corn and the control. In addition, environmental interactions data was collected in the phenotypic studies (plant-insect, plant-disease, and plant environment interactions) and studies on abiotic stress tolerance, potential for the crop to volunteer, and survival outside of cultivation. The results from the environmental interactions assessments also support the conclusion that MON 87460 corn has no increased susceptibility or tolerance to specific diseases, arthropods, or abiotic stressors, with the exception of improved drought tolerance. Finally, MON 87460 corn was not altered in its ability to volunteer in cultivated fields or survive in areas not managed for agricultural production compared to conventional corn.

Taken together, these data support a conclusion that MON 87460 corn poses no increased plant pest potential, including weediness potential, and no adverse environmental impact compared to conventional corn.

MON 87460 Corn will not Adversely Affect Non-Target Organisms including those Beneficial to Agriculture

The environmental assessment of MON 87460 corn demonstrates that it poses negligible risk to non-target organisms. The assessment took into consideration the familiarity with CSPB and NPTII protein modes of action and their expression levels in MON 87460 corn. Although neither CSPB nor NPTII are known to exert any effects on pest and non-pest organisms, studies were conducted to examine the potential effects of MON 87460 corn on organisms (biotic stressors) that interact with and may affect corn. Studies demonstrate a lack of any effects observed in various species exposed to MON 87460 corn.

During the U.S. and Chile phenotypic field studies at 31 locations in 2006 and 2007, each field site was rated at four time intervals during the season for specific insects (pest and non-pests), and diseases. The purpose of these observations was to assess whether the plant-disease or plant-insect interactions of MON 87460 were altered compared to commercial corn. Twenty-one pest and non-pest arthropod categories (species or group) and 19 disease categories were evaluated. Of the more than 388 pest and non-pest arthropod evaluations, only two differences were observed between MON 87460 and the control. The differences detected were either within the range of the references, or were isolated to a single study-site location. The few differences

detected were small in magnitude, did not represent a trend in the data, and are not considered to be biologically meaningful in terms of potential impact on non-target organisms.

Cultivation of MON 87460 Corn is not Expected to have Adverse Effects on Agronomic Practices or Land Use

An assessment of current corn agronomic practices confirmed that the introduction of MON 87460 corn is no more likely to impact land use, cultivation practices, or the management of weeds, diseases, and insects than the use of conventionally bred drought tolerant corn. Seed production, crop rotation and tillage practices, or weed, disease, and insect management practices are not expected to be altered following the introduction of MON 87460 corn, which is environmentally benign and a beneficial additional tool for corn growers in areas suitable for commercial corn production, but prone to frequent drought stress.

Conclusion

As presented above, all available data and information demonstrate the familiarity of MON 87460 corn as compared to conventional corn and, moreover, show that MON 87460 corn has no greater weediness or plant pest potential. This conclusion is based on multiple, well established lines of evidence including the biology of corn and a comprehensive assessment that spans the entirety of MON 87460 corn from the molecular level to the whole crop level. MON 87460 corn is as safe as conventional corn for use as food and feed and poses no more environmental risk than conventional corn.

Published Literature

Links to published literature on MON 87460 corn and the function of the CSPB protein are provided below.

Castiglioni, P., D. Warner, R.J. Bensen, D.C. Anstrom, J. Harrison, M. Stoecker, M. Abad, G. Kumar, S. Salvador, R. D'Ordine, S. Navarro, S. Back, M. Fernandes, J. Targolli, S. Dasgupta, C. Bonin, M.H. Luethy and J.E. Heard. 2008. Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. Plant Physiology 147:446-455.
link

Chang, J., D.E. Clay, S.A. Hansen, S.A. Clay and T.E. Schumacher. 2013. Water stress impacts on transgenic drought-tolerant corn in the northern great plains. Agron. J. 106:125-130.
link

Harrigan, G.G., W.P. Ridley, K.D. Miller, R. Sorbet, S.G. Riordan, M.A. Nemeth, W. Reeves and T.A. Pester. 2009. The forage and grain of MON 87460, a drought-tolerant corn hybrid, are compositionally equivalent to that of conventional corn. Journal of Agricultural and Food Chemistry 57:9754-9763.
link

Nemali, K.S., C. Bonin, F.G. Dohleman, M. Stephens, W.R. Reeves, D.E. Nelson, P. Castiglioni, J.E. Whitsel, B. Sammons, R.A. Silady, D. Anstrom, R.E. Sharp, O.R. Patharkar, D. Clay, M. Coffin, M.A. Nemeth, M.E. Leibman, M. Luethy and M. Lawson. 2015. Physiological responses related to increased grain yield under drought in the first biotechnology-derived drought-tolerant maize. Plant, Cell and Environment 38:1866–1880.
link

Sammons, B., J. Whitsel, L.G. Stork, W. Reeves and M. Horak. 2014. Characterization of drought-tolerant maize MON 87460 for use in environmental risk assessment. Crop Science 54:719-729.
link