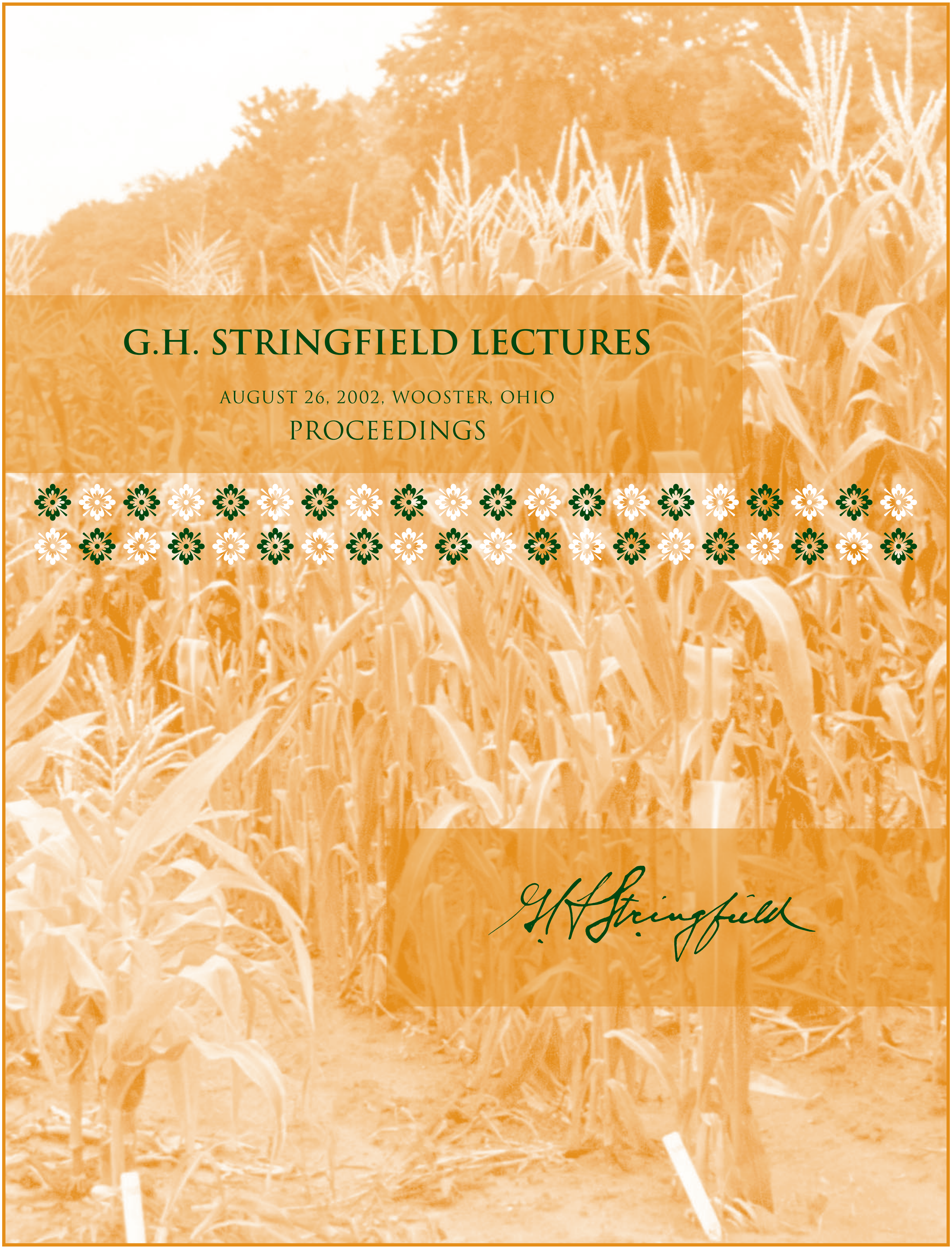
G.H. STRINGFIELD LECTURES

AUGUST 26, 2002, WOOSTER, OHIO

PROCEEDINGS



On the occasion of the inaugural Stringfield Lectures, many friends and colleagues gathered to learn about the life and work of Glen Stringfield, and to honor Dr. William R. Findley, the first recipient of the G.H. Stringfield Award. The award was made on behalf of The Ohio State University Department of Horticulture and Crop Science and the Ohio Agricultural Research and Development Center for outstanding contributions to the science of corn breeding and genetics.

Dr. William R. Findley was born in Manhattan, Kansas on June 26, 1920. He served in the Pacific Theater during WWII as a navigator on B17 and B24 bombers. He attended Kansas State University and earned the B.S. and M.S. degrees in Agriculture with majors in genetics. His career began with the USDA in 1950 at the Plant Industry Station in Beltsville, Maryland in corn breeding with Dr. Merle T. Jenkins. Research focused on developing corn lines resistant to northern corn leaf blight and determining the inheritance of host resistance. Dr. Findley was also a part-time Ph.D. student at the University of Maryland, and after completing the degree, he accepted a transfer to Kansas State University to direct the corn breeding program. In 1960, he joined the OSU-ARS cooperative corn breeding program at the OARDC. The initial focus was on leaf blights and European corn borer resistance, but in 1963 corn viruses infected several thousand acres of corn in southern Ohio, and battling the viral diseases became his focus until he retired in 1985.

We gratefully acknowledge the generous support of Mr. and Mrs. Cecil Robinson; The Andersons, Inc., of Maumee, Ohio; and the contributors to The Wooster Lecture Series OARDC development fund for making the lectures and the award possible.

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Edited by R.C. Pratt

Below are views of Glen Stringfield's field research conducted while he was an M.S. student. His thesis, published in 1927, was titled "Types of Plot and Field in Comparative Field Crop Tests." His thesis adviser was Dr. J.B. Park from the OSU Columbus campus. The research was conducted with oats and wheat.

Three rod plots of oats in 1 ft. and 3 ft. widths were planted in replicated trials. The research showed a reduction in the standard deviation of yield values with increased plot width.

A view of field plot research performed in 1926 with 1/100-acre plots. In the foreground, paired plots of Miami, Ohio201, and Fulghum oats can be seen in continuous series. In the background, 1/100 acre plots of Miami oats have been planted in differing lengths and widths.

The G.H. Stringfield Lectures

An introduction to our invited lecturers:

Dr. Forrest Troyer – The name Troyer is certainly well known to all of us in the Wayne-Holmes County area. Dr. Troyer mentioned that bringing a Troyer to Wooster is about like carrying coal to Newcastle. He said that time would tell if it was worth the trouble, and it certainly was. Two of Dr. Troyer's grandparental families lived in Holmes County. He related an interesting story about his grandfather David who was done in by the Panic of 1893. He shipped a boxcar of onions to Pittsburgh and received a telegram stating their sale did not cover the freight charge. His offer to send more onions was declined. David Troyer sold the farm, and started walking westward. He wound up buying farmland in the Mississinnewa River Valley in Indiana to raise corn and fatten hogs. That's where Forrest Troyer was born and raised.

Dr. Troyer is a graduate of the University of Minnesota. He was a highly successful corn breeder — developing or co-developing 41 commercial hybrids during his career with Pioneer Hi-Bred International and Dekalb Genetics. Dr. Troyer has published and presented many scholarly works and is a fellow of the American Association for the Advancement of Science, the American Society of Agronomy, and the Crop Science Society of America. His three most recent awards are the Agronomic Service Award, the National Award for Agricultural Excellence from the National Agricultural Marketing Association, and the Minnesota Outstanding Achievement Award. Dr. Troyer is also an authority on the history of Corn Belt maize. He shared with us his avocation as a historian and did a wonderful job informing us of the life and work of the namesake of the G.H. Stringfield Award.

Dr. Michael McMullen – Dr. McMullen also has roots in Ohio. He grew up in the Cincinnati area and attended Miami University. Dr. McMullen received his Ph.D. in genetics from the University of Chicago in 1981. After completing post-doctoral appointments at the University of Washington and the University of Minnesota, he began his career with the USDA-ARS as a research geneticist and member of the OSU-ARS Maize Virus Program at the Wooster Campus in 1986. During his time at Wooster, he focused on genome structure of maize viruses and the genetic basis of viral resistance in maize. He is presently an ARS maize geneticist at the University of Missouri-Columbia. As a co-principal investigator on the National Science Foundation sponsored Maize Mapping Project, he is working toward the goal of developing an integrated genetic/physical map for maize. Current research interests also include understanding the genetic basis of agronomic traits. He has worked extensively on the relationship of quantitative trait loci to biochemical pathways through the study of the synthesis of flavones in maize silks, and their relationship to resistance to the corn earworm. Dr. McMullen is recognized as an insightful researcher with broad interests and talents. He has provided leadership to the maize genetics research community through the National Research Initiative and the NSF sponsored maize mapping project. The maize genome database has greatly enhanced the utilization of maize genetic maps and mapping resources. He also has contributed to organization of the Annual Maize Genetics Conference.

Summaries of the lectures are presented below:

"G.H. Stringfield"

Forrest Troyer
Cargill Hybrid Seeds (retired)
DeKalb, Illinois

Glen Stringfield grew up in the far southeastern corner of Nebraska. He was a student of Dr. Kiesselbach, a renowned corn scientist. 'String,' as he was commonly known, graduated at the top of his baccalaureate class at the University of Nebraska in 1924. He conducted his M.S. research at OSU and completed his thesis in 1927 working on field research plot techniques. He went on to develop excellent, widely grown maize inbreds and hybrids and contributed useful articles on maize breeding to professional journals.

During his lifetime he became a member of Alpha Zeta and Gamma Sigma Delta honorary societies. He was elected a Fellow of the American Association for the Advancement of Science, the American Society of Agronomy, and the American Genetics Society. He was inducted into the First Ohio Agricultural Hall of Fame in 1966 and he received the National Council of Commercial Plant Breeders Award in 1968.

Glen Stringfield's graduate research was published in a timely manner (Stringfield, 1928) and he continued throughout his career to communicate information and ideas to a broad audience. A paper on improving inbred lines by backcrossing (Stringfield, 1951) systematically and completely explained the essence of backcrossing. This paper could be helpful now with so much inconsistent backcrossing of transgenic traits in the seed corn industry. This paper helped develop my breeding philosophy. I usually select in backcross populations.

Stringfield's paper on 'Heterozygosity and hybrid vigor in maize', published in 1950, compared the average of the two possible first backcrosses with the F_2 for a four-inbred diallel set of six single-cross hybrids. This provides average identical gene frequencies for the two (BC_1 and F_2) types of populations. He found the average of the two backcrosses (12 genotypes) to be significantly higher yielding than the average of the F_2s (six genotypes). String states: "Inbreds which survive the selection procedure may tend to have favorable combinations of genes. A favorable combination might contribute more vigor than a random assortment of genes individually just as good." My breeding philosophy is to maintain and improve desirable genetic linkages. Linkage is my friend. I suggest that selection for what Stringfield calls favorable combinations of genes (i.e. within-inbred epistasis), is important and is increasing over time as average yield increases (Troyer and Rocheford, 2002).

His selection program at the Ohio Agricultural Experiment Station developed the notable inbreds Oh07, Oh26, Oh43, and Oh51A. (Descriptions of inbreds may be found in the Stringfield (1959) publication; available by request directed to R. Pratt). He was a ruthless discarder; he ran a clean, relatively lean program. On a group tour of federal corn breeding stations, while at Mississippi State he was asked for advice to improve the large program. String answered, "Frankly, I would throw 90% of this stuff away." Following WWII, in a period of military atmosphere, String characterized Iowa inbreds as coming to Ohio to get medical discharges (Stringfield, 1964). A peculiarity of String's program was to cross new inbreds to their parents and yield test for additive gene action. Much of his effort was on tolerance to northern and southern leaf blight, and to European corn borer.

Glen Stringfield retired after 30 years at Wooster and joined DEKALB AgResearch in 1959. Colleagues at DEKALB felt String had a green thumb, but apparently did not soon benefit from his "broad-line" concept (Stringfield, 1972). Apparently String felt it was important to balance offense and defense using the broad-line concept and he developed no homozygous inbreds at DEKALB. Some colleagues at DEKALB felt "Over time the broad-line concept proved to be a good form of population improvement. Good homozygous inbreds were developed from Stringfield's materials. And the broad-line hybrid concept might have worked a decade sooner (before single-cross hybrids) if only one parent were a broad-line." String was a developer (with Henry Slade and others) of the single-cross hybrid DELALB XL45. The hybrid was first grown commercially in 1963 and it rapidly became popular in the northern U.S. Corn Belt. XL45 was the right hybrid at the right time. It was widely adapted — grown from Minnesota to Texas and from Colorado to Delaware. Sales grew rapidly to exceed one million bags annually from 1967 through 1970. Total sales were about 6.7 million bags over a 13 year period. See Roberts (1999) for a fascinating story of XL45 development (Troyer, 1996).

String enjoyed talking corn breeding with other breeders. He used straightforward body language when listening to an adversary's argument. He typically held his lit and smoking pipe on the table in front of him, stared at the bridge of the speaker's nose, and moved his head in wide swings — forward and backward if he agreed, and from side to side if he did not. String wasn't satisfied to end a discussion with a stand-off, he much preferred to come to a mutual conclusion — the conclusion he started with!

Glen Stringfield was a determined traditionalist and a Cleveland Indians fan. He helped hand-plant the nursery every year, comparing it to a baseball player's hitting streak. It was a source of pride. String passed away on June 28, 1975 while listening to a Cleveland Indians baseball game.

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"Quantitative Trait Locus Analysis and Gene Discovery in Maize"

Michael D. McMullen
USDA-ARS, Plant Genetics Research Unit, and Plant Sciences Unit, University of Missouri, Columbia, MO

The past fifteen years have seen an explosion of information on the structure, organization, and functions of the maize (*Zea mays* L.) genome, including the development of high-density molecular marker maps. One application of new mapping technologies has been the genetic dissection of quantitative traits with much greater precision than was previously possible (Paterson, 1998). Still, the quantitative trait loci (QTLs) detected are generally rather poorly defined regions, and the size of a QTL's phenotypic effect is sometimes confounded with its location relative to the nearest marker or to a nearby QTL. For most traits, genetic and biochemical information on metabolic pathways is extremely limited, and therefore, it is difficult to interpret QTL results in terms of regulatory and structural genes, duplicate function loci, feedback inhibition, branched pathways or other phenomena affecting trait expression. We have exploited a unique research system to analyze the genetic control of a quantitative trait of economic importance (antibiosis to the corn earworm) and to interpret the results in terms of the well-characterized flavonoid and phenylpropanoid pathways. From these studies we have documented a number of fundamental mechanisms for the genetic control of quantitative traits.

The corn earworm, *Helicoverpa zea* (Boddie), is a major insect pest of maize in the United States and elsewhere in the Western Hemisphere. Corn earworm eggs are laid on the silks and the larvae access the ear by feeding through the silk channel. Host-plant resistance to corn earworm by antibiosis is due to the presence of the C-glycosyl flavones maysin, apimaysin, methoxymaysin and related compounds in maize. Upon ingestion by corn earworm, the flavones are believed to be oxidized to quinones, which bind amino acids making them unavailable and thus inhibiting larval growth. In addition, some compounds in the general phenylpropanoid pathway such as chlorogenic acid (3-caffeolyquinic acid) also exhibit antibiotic activity against the corn earworm.

Flavonoids are a widely distributed class of plant phenolic compounds whose diverse functions include pigmentation, disease and insect defense, growth regulation, protection against UV-radiation, and maintenance of pollen tube growth. One major section of the flavonoid pathway leading to the synthesis of C-glycosyl flavones (including maysin and related insect resistance factors), phlobaphenes (responsible for red cob and pericarp pigments), and 3-deoxyanthocyanins is regulated by the *p1* (*pericarp color1*) locus. Another section of the pathway leads to anthocyanins and is regulated by the coordinate action of either *r1* (*colored1*) + *c1* (*colorless1*) or *b1* (*colored plant1*) + *pl1* (*purple plant1*) in a tissue-specific manner. *p1*, *c1*, and *pl1* all have *myb*-homologous DNA binding domain sequences[1], but, whereas *p1* acts alone to activate transcription of structural genes under its control, *c1* and *pl1* require *r1* and *b1* for activity. In addition to the regulatory genes many of the enzyme-encoding genes of the maize flavonoid pathway have been cloned and characterized allowing us to design experiments to characterize the genetic effects caused by variation at specific pathway steps.

Our approach has been to conduct QTL analysis on multiple populations specifically designed to segregate for different steps in the flavone pathway or to differ in amounts or type of flavone produced. Our results have demonstrated; i) the importance of regulatory loci as QTLs, ii) the presence of a major QTL from chromosome 9S with essentially recessive gene action for high maysin, iii) the importance and biological basis of epistasis for QTL in the flavone pathway, iv) the importance of the interaction of connecting pathways in determining end product levels, and v) consistent QTLs for chlorogenic acid synthesis on chromosomes 1 and 2.

We have observed the correlation between phenotypically different *p1* alleles and a major QTL at its position in at least ten populations from different crosses. The identity of *p1* as the QTL for flavone synthesis has recently been confirmed by transformation experiments (Grotewold et al. 1998, Bruce et al. 2000). This result demonstrates the importance of transcription factors as QTL for maysin synthesis and corresponding earworm resistance. A consistent finding by our group is that the *p1* locus often exhibits major epistatic interactions with other loci. In multiple populations we have detected a second major QTL for maysin synthesis on the short arm of chromosome 9 (Byrne et al. 1996, 1998). We have designated this QTL (gene) *rem1* (*<u>r</u>ecessive <u>e</u>nhancer* of *<u>m</u>aysin1*). The *rem1* gene effect requires a functional *p1* allele, and in populations that segregate for variation at both *p1* and *rem1*, *rem1* exhibits epistatic interaction with *p1*. Understanding the basis of the *rem1* effect on the flavone pathway is currently a focus of the laboratory.

The *a1* (*anthocyaninless1*) locus in maize encodes NADPH dihydroflavonol reductase. This enzyme is required for the synthesis of 3-deoxyanthocyanins in silks, but is not required for flavone synthesis. We demonstrated that in a population established for segregation for variation at *p1* and *a1*, both loci were major QTLs and exhibited a highly significant epistatic interaction. In conjunction with functional alleles at *p1*, recessive *a1* alleles shunted intermediates to the flavone pathway increasing maysin levels (McMullen et al., 2001).

Shunting of intermediates between pathways was also established as the basis of QTL effects for chlorogenic acid synthesis. We demonstrated that the combination of functional *p1* alleles to induce the flavone pathway together with nonfunctional alleles at the

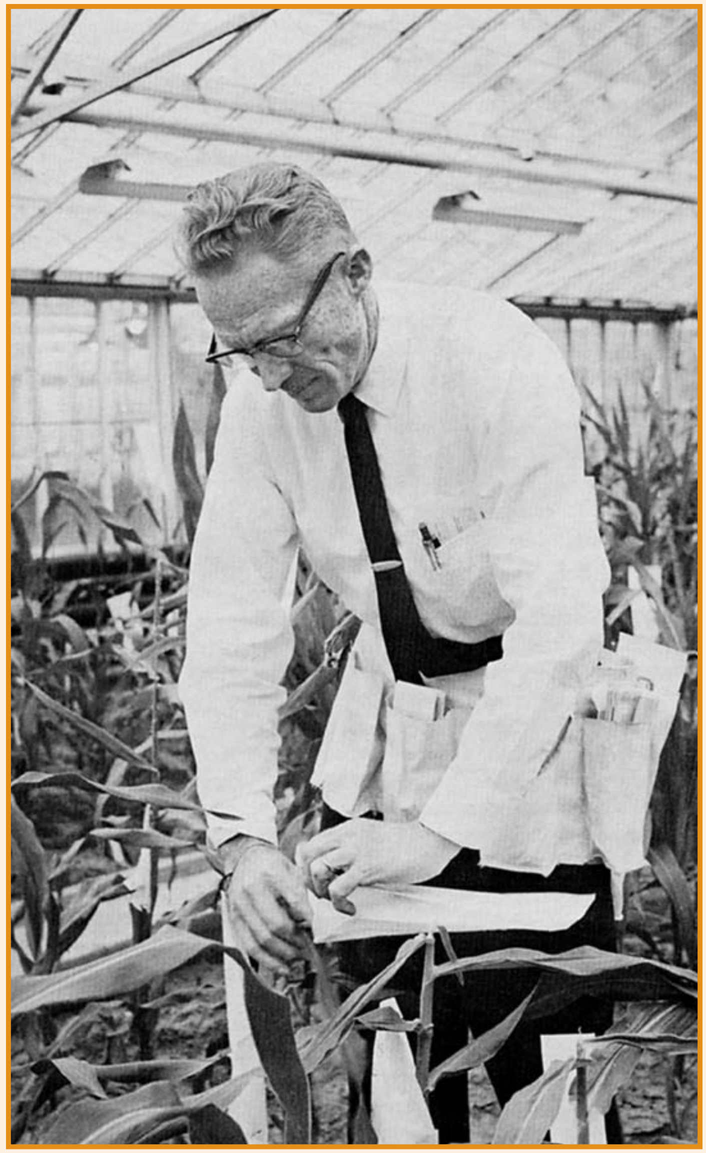
[1]Transcription factors containing the myb-homologous DNA binding domain are widely distributed in eukaryotes. Myb proteins are involved in the complex control of phenylpropanoid and flavanoid biosynthesis. The myb-domain proteins appear to play a role in the interaction of plants with the environment, providing plasticity in plant metabolism and development.

two chalcone synthase genes in maize, *c2* (*colorless2*) and *whp1* (*white pollen1*), resulted in a major increase in the accumulation of chlorogenic acid.

We have also established the relative role of *c2* and *whp1* in flavone synthesis. There exists a dosage-dependent relationship between *c2* and *whp1* in which *whp1* acts cooperatively with functional *c2* to increase flavones and partially compensates for nonfunctional *c2*. A major difference exists in the gene action between these duplicate loci in that *c2* requires functional *p1* to increase maysin, but *whp1* does not. This result indicates that while *p1* directly regulates *c2*, *whp1* is under separate regulatory control. This is manifested in the QTL effects as an epistatic interaction that is evident between *p1* and *c2*, but not between *p1* and *whp1*.

We have studied three populations with the goal of understanding high chlorogenic acid synthesis in maize silks. Two loci, *p1* on chromosome 1 and a QTL on chromosome 2 that we have named *qtl2* (*QTL for chlorogenic acid:chromosome2*), are the major loci required for high chlorogenic acid levels. With this information, we can design maize lines with chlorogenic acid as the silk resistance mechanism.

By combining a candidate gene approach with QTL analysis we have made major progress in understanding the genetic basis of maysin synthesis and the associated antibiotic resistance to corn earworm. The results have led to improved selection strategies for maize breeders, enhanced understanding of the flavonoid pathway, and indirectly to a better understanding of the genetic mechanisms underlying quantitatively inherited characters.



Our good friend Bill Findley is surely remembered as the best-dressed corn pollinator of all time!

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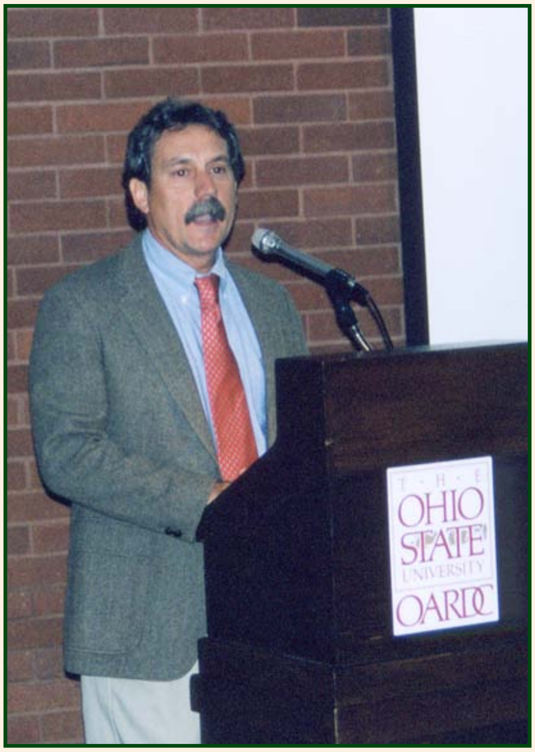
LEFT OARDC Associate Director Dr. Dave Benfield (left) presenting the first G.H. Stringfield Award in recognition of outstanding contributions to the science of corn breeding and genetics to Dr. Bill Findley.

RIGHT Sponsor Mr. Cecil Robinson (right) visits with retired OARDC Associate Director Dr. Russ Conrad (left) during the reception.



ABOVE Dr. Mike McMullen, USDA/ARS Research Geneticist at the University of Missouri, presented a lecture entitled "Quantitative Trait Locus Analysis and Trait Discovery in Maize."

ABOVE Invited lecturers (from left) Dr. Forrest Troyer and Dr. Michael McMullen, G.H. Stringfield award recipient Dr. Bill Findley, and OSU maize breeder Dr. Rich Pratt pause for a photograph.

LEFT Bill Findley's daughters Gloria and Barb, and their husbands Paul Wirt and Cliff Rydell, enjoy a pleasant conversation at the dinner table. (They're probably noting that just as Glen Stringfield did, their father keeps swapping pollen bags long after retirement.)

Maize virus researchers organized host resistance research in cooperation with farmers, seedsmen, and extension agents in southern Ohio starting in the 1960s. Left, one can see the extreme virus susceptibility typical of corn germplasm before systematic breeding was conducted. The results of the collaborative efforts can be seen to the right in the resistant germplasm that was selected.