

**PROGRESS REPORT**  
to  
**NORTH CAROLINA PEANUT GROWERS ASSOCIATION, INC.**

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2012

**Project Title:** Breeding Peanuts for Multiple Disease Resistance

**Project Investigator(s):** Thomas G. Isleib  
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**Summary:** This project accelerates the process of development of multiply disease-resistant virginia-type peanut cultivars. Each year a new set of crosses is made, usually a set of agronomically good parents crossed with another set with the most disease resistance available. The program uses a winter seed nursery in Puerto Rico to achieve two generations per calendar year, and it uses early generation testing to identify the families most resistant to the four diseases that commonly have economic impact in North Carolina: early leaf spot caused by *Cercospora arachidicola*, *Cylindrocladium* black rot caused by *C. parasiticum*, *Sclerotinia* blight caused by *S. minor*, and tomato spotted wilt caused by the *Tomato spotted wilt tospovirus* (TSWV). We grow separate tests for the four diseases in trials called the "Disease Selection Test (DST)" series, each specially managed to promote a specific disease, but we score other diseases as opportunity allows. In each trial we grow the same set of families.

In 2012 we had 20  $F_{2:4}$  (selected as  $F_2$  plants two generations removed from the cross, tested as  $F_4$  grand-progeny of those selected plants), 84  $BC_1F_{4:6}$ , and 83  $F_{6:8}$  families. All of these families were either high oleic or carrying the gene for the high oleic trait. We consider the  $F_{6:8}$  families to be genetically stable "breeding lines," and we select the best among them but not within them. For the  $F_{2:4}$  and  $BC_1F_{4:6}$  family types, we selected good looking plants within the 20% most disease-resistant families, sent the progeny to the winter nursery, and will return enough seed to have our replicated trials and a selection nursery in North Carolina in 2013. In addition to the disease evaluations, the  $F_{6:8}$  families were tested in replicated trials for yield and grade at two locations in North Carolina.

Once a line has been tested for disease reaction as an  $F_{6:8}$  family, we "graduate" resistant families into our ongoing program of evaluating lines for reactions to the four diseases, our "Disease Advanced Line Test (DAT)" series, or we graduate high-yielding lines into our Advanced Yield Test (AYT) with yield trials conducted at three locations in North Carolina and leading to the multi-state Peanut Variety and Quality Evaluation program and ultimately to cultivar release. Most years there is some overlap of the sets of lines graduated to the DAT and AYT. Because we had not collected yield data on  $F_{6:8}$  families in 2011, we graduated all of them to the 2012 AYT. Only four graduated to the 2012 DAT. We moved other  $F_6$ -derived families from previous years' testing forward in the DAT and AYT programs. Note that we do test disease reactions of lines in the AYT starting in the second year of AYT testing, and that lines in the DAT can move into the AYT program if their performance so warrants.

Bailey was released from this program in 2008, Sugg in 2009. We did not release a new cultivar in 2012. The area's shellers do not want to absorb a new cultivar every year, preferring them to come out every three to five years. We intend to replace Bailey with one of the high-oleic versions once we have collected sufficient disease, yield and grade data to warrant release. In the meantime, a number of lines that have passed through the NPB-funded program will have been sufficiently tested by the end of the 2012 season, and a release will be made if the data support it. Our ultimate aim is to replace all the cultivars currently on the seed market with high-oleic, disease-resistant releases.

## REPORT

**Project Title:** Breeding Peanuts for Multiple Disease Resistance

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**Objectives:** Develop high-oleic peanut cultivars with resistance to CBR, Sclerotinia blight, TSWV, and early leafspot using early generation selection augmented by a winter nursery.

**Procedures:** A new set of crosses was made in the greenhouse on the NCSU campus in 2012: a factorial mating between a set of six agronomically “good” parents (N09037oI, N09039oIF, N09042oIF, N10046oI, N10047oI and N10053oI) and a set of six highly disease resistant parents selected from an earlier cycle of the program. The  $F_1$  seeds were planted in the 2012-2013 winter seed nursery at Juana Diaz, PR (PRWN) in November.

The  $F_2$  seeds produced at the 2011-2012 PRWN from 25 crosses made in 2011 were planted at the NCDA&CS Peanut Belt Research Station (PBRS) in Bertie Co. at Lewiston, NC, in the spring of 2012. After digging in October, individual plant selections were made on the basis of pod appearance, and 130  $F_{2:3}$  progenies of selected plants were planted at the 2012-2013 PRWN in November.

The corresponding 20  $F_{2:3}$  progenies from the 2011-2012 PRWN were harvested in March 2012 by first collecting a single pod from each plant from which a single seed was planted for selection in the  $F_{2:4}$  Nursery, Accelerated at PBRS (12 L F4A). After performing “single seed descent” (SSD), each 2011-2012 PRWN  $F_{2:3}$  plot was harvested in bulk to provide sufficient seed to allow for replicated testing resistance to the four diseases at sites specially chosen and managed to promote development of diseases: Sclerotinia blight, leaf spot and CBR on infested fields left untreated with the protectant fungicides used to control them, TSWV in a trial in which plants were spaced 20 inches apart and left untreated with insecticides that would reduce the population of the thrips that vector the virus. Once we identified the four “best”  $F_{2:4}$  families on the basis of DST data, individual plant selections were made within those families in the nursery planted for the purpose at PBRS from the 2011-2012 PRWN  $F_{2:3}$  single-seed descent harvest.  $F_{4:5}$  progenies of 16 selected plants were planted at the 2012-2013 PRWN in November.

The 84  $F_{4:5}$  progenies from the 2011-2012 PRWN were harvested in March 2012 with both SSD and bulk harvest as previously described for the  $F_{2:3}$  families. The single seed per plant was planted in May in the 2012  $F_{4:6}$  Nursery, Accelerated at PBRS (12 L F6A); the bulk-harvested seed was planted in the 2012 DST series. Once we identified the 17 “best”  $F_{4:6}$  families on the basis of DST data, individual plant selections were made within those families, and  $F_{6:7}$  progenies of 68 selected plants were planted at the 2012-2013 PRWN in November.

The 83  $F_{6:7}$  progenies from the 2011-2012 PRWN were harvested in March 2012 with only bulk harvest. We consider  $F_6$ -derived families to be sufficiently genetically stable and uniform that we classify them as “breeding lines” and do no further within-family plant selection. The bulk-harvested seed was planted in the 2012 DST series and the 2012 Disease Preliminary Line Nursery (12 L DPN). They were also tested in replicated preliminary trials for yield and grade at PBRS and at the Upper Coastal Plain Research Station (UCPRS) in Edgecombe Co. near Rocky Mount, NC.

We had separate tests for four diseases with 196 entries in each (20  $F_{2:4}$ , 84  $BC_1F_{4:6}$ , 83  $F_{6:8}$  families, and 9 cultivars and checks). The plots at PBRS for early leaf spot caused by *Cercospora arachidicola* were not provided with any fungicidal spray. In late September we rated defoliation on the

plots using a proportional scale of 1 (representing no defoliation) to 9 (complete defoliation usually accompanied by death of the plants). In spite of having a good environmental conditions for development of leaf spot, very little spotting was found in the field prior to October, and there was very little defoliation. The plots at PBRs for tomato spotted wilt caused by the *Tomato spotted wilt tospovirus* received no insecticide treatment to manage the thrips that vector the virus and were planted at 20-inch (50 cm) seed spacing to maximize thrips feeding and TSWV incidence. The plots at UCPRS for *Cylindrocladium black rot* (CBR) caused by *C. parasiticum* were not treated with metam sodium fumigant nor were the seeds treated with prothioconazole to control CBR. The field has a history of CBR, was rotated between peanut and soybean, an alternate host of CBR, and the late part of the season was cool and moist, ideal for development of CBR. Yet no CBR symptoms were found in the plots. Plots at UCPRS for *Sclerotinia blight* caused by *S. minor* were not treated with fluazinam or boscalid to control blight. All DST trials were conducted in single rows in 14x14 simple square lattice designs (two replicates with incomplete blocks to allow for variation within replicates). For CBR, SB, and TSWV, emerging plants were counted approximately one month after planting, then symptomatic plants were counted at appropriate times during the season, and disease incidence was expressed as a proportion of emerged plants for purposes of statistical analysis.

After analyzing the data for all four diseases, mean disease reaction values for the families were expressed on a zero-to-one scale with a score of zero for the mean indicating the greatest level of susceptibility and a score of one indicating the most resistant family. We calculated the arithmetic mean for each family across the four diseases and identified the best 20% of the F<sub>2:4</sub> families, F<sub>4:6</sub> families, and F<sub>6:8</sub> families. We also identified the best 20% of all families. We selected the best looking plants in the F4A, and F6A nurseries at PBRs from within the families identified as "best."

We have the option of "graduating" resistant families into our ongoing program of evaluating lines for reactions to the four diseases, our "Disease Advanced Line Test (DAT)" series, or of graduating high-yielding lines into our Advanced Yield Test (AYT) with yield trials conducted at three locations in North Carolina and leading to the multi-state Peanut Variety and Quality Evaluation program and ultimately to cultivar release. Most years there is some overlap of the sets of lines graduated to the DAT and AYT.

All families currently in the program are either have the University of Florida's patented "high oleic trait, *i.e.*, elevated content of oleic fatty acid in the seed oil leading to extended shelf life for high oleic peanuts or products made from them, or they carry the recessive gene for the high oleic trait. In order to identify plants with the trait, we perform a progeny test of all F<sub>2</sub> selections, of all F<sub>4</sub> selections that derive from heterozygous F<sub>2</sub> plants, and of all F<sub>6</sub> plant selections that derive from heterozygous F<sub>4</sub> plants. By the time we have genetically stable F<sub>6</sub>-derived breeding lines, they are high oleic. We use gas chromatography to assess five progeny from each tested selection. If the plant is normal oleic (homozygous for the "normal" allele), it will produce all normal plants with oleic-to-linoleic (O/L) ratios up to slightly more than two. Likewise a high oleic selection (homozygous for the high oleic gene) will produce all high oleic progeny. If the selection is heterozygous, then its self-pollinated progeny will occur in a 1:2:1 ratio of normal to heterozygous to high oleic seeds, and we will be able to discern it. There is a 1/1024 probability that all the progeny will be normal oleic, and a 1/1024 probability that they will all be high oleic, totaling a 1/512 probability that we reach an erroneous conclusion. We consider this roughly 0.2% error rate to be acceptable. We do make a final purification of any breeding that survives the testing program and will be released. We assess the fatty acid genotype of a number of plants taken from the line and pool only the high oleic plants.

**Results and Discussion:** Because we had not collected yield data on  $F_{6:8}$  families in 2011, we graduated all of them to the 2012 AYT. Only four graduated to the DAT. We moved other  $F_6$ -derived families from previous years' testing forward in the DAT and AYT programs. We perform an annual summary of disease reactions and yield using databases maintained for this purpose (Fig 1.). Note that we do test disease reactions of lines in the AYT starting in the second year of AYT testing, and that lines in the DAT can move into the AYT program if their performance so warrants.

The DAT series had 56 common entries tested for the three diseases that were measurable in 2012 including seven  $BC_1F_{6:11}$  families from the 2010 AYT, two  $F_{6:10}$  families from the 2010 DPT, one  $BC_1F_{6:10}$  family from the 2009 DPT, six  $F_{6:10}$  families from the 2010 DPT, four  $BC_1F_{6:9}$  families from the 2011 DPT, and five lines entered upon the request of Dr. Roy Pittman, the former USDA-ARS peanut germplasm curator, and 18 checks including released cultivars (NC-V 11, Gregory, Perry, Phillips, Bailey, Sugg, CHAMPS, Florida Fancy, and runner-type cultivar Florida-07) and disease-resistant lines (Georgia Green, GP-NC 343, NC 3033, N96076L, GP-NC 343, PI 109839, PI 121067, PI 269685, PI 270806, and PI 576636). Some of these lines originated in the DST program but fell out of the accelerated program because they did not exhibit sufficient resistance to be retained. Such families enter the conventional cultivar development stream that achieves only one generation per year and in which selection and retention in the program are based solely on pod characteristics, yield and grade. Of the 33 experimental lines developed by the breeding program rather than the species program, 18 were also entered in the multiple-location Advanced Yield Test series for broader evaluation of yield and grade, and one was entered in the multiple-location Early Maturity Advanced Test.

Disease data from the 2012 season on preliminary and advanced  $F_6$ -derived lines was combined with data collected from 2000-2011, and means adjusted to a common environmental effect were computed. Adjusted means were converted to a zero (worst) to one (best) scale for each of the four diseases, and an arithmetic mean disease score was computed. All diseases were weighted equally. Similarly, an adjusted mean yield was computed using all data collected from plots with conventional disease management from 2000 through 2012. Of the 20 best lines for disease resistance, 14 came from the accelerated resistance selection program (Fig. 1), all of them high-oleic selections from the 2007 through 2012 DPT. Although it is often the case that the most disease-resistant lines are not the highest yielding or have the best grade, many of these selections yielded extremely well. The top 20 disease resistant lines included Bailey, released in 2008 and now widely available to growers as certified seed. The group also included three high oleic Bailey backcross derivatives and one high oleic backcross derivative of Bailey sister line N03090T. The commercial value of the experimental selections cannot be known with certainty until they have passed through the multiple-year multiple-location testing program, but several of them appear to combine high yield with good disease resistance. One of Dr. Tallury's lines, SPT 07-01, combined outstanding disease resistance with high yield based on the data available to date. It must be noted that means based on more replication are more reliable, means based on less replication less so.

Bailey was released from this program in 2008, Sugg in 2009. We did not release a new cultivar in 2011. The area's shellers do not want to absorb a new cultivar every year, preferring them to come out every three to five years. We intend to replace Bailey with one of the high-oleic versions once we have collected sufficient disease, yield and grade data to warrant release. In the meantime, a number of lines that have passed through the NPB-funded program will have been sufficiently tested by the end of the 2012 season, and a release will be made if the data support it. Our ultimate aim is to replace all the cultivars currently on the seed market with high-oleic, disease-resistant releases.

## 2012 Yield versus disease resistance

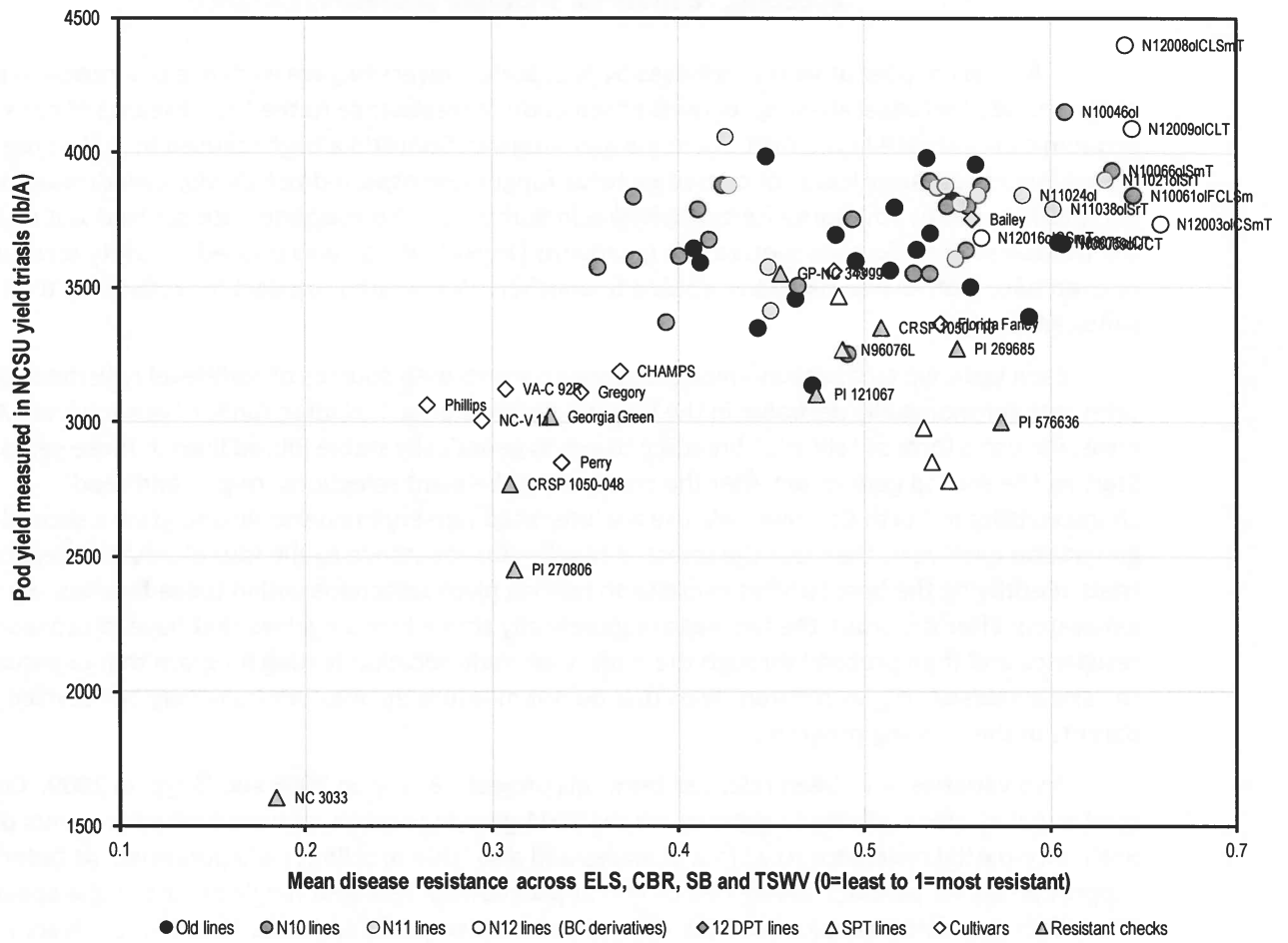


Figure 1. Pod yield with disease control versus arithmetic disease resistance index (mean of three disease scores adjusted to a scale of 0=worst to 1=best).

## Lay Interpretation of Results Breeding Peanuts for Multiple Disease Resistance

A series of peanut variety releases by N.C. State University gave evidence of a problem in our program. We had separate subprograms of selection for resistance to the four diseases of consistent economic importance in the North Carolina and Virginia: Sclerotinia blight caused by soil-borne fungus *Sclerotinia minor*, early leaf spot caused by foliar fungus *Cercospora arachidicola*, *Cylindrocladium* black rot (CBR) caused by soil-borne fungus *Cylindrocladium parasiticum*, and tomato spotted wilt caused by the thrips-vectored *Tomato spotted wilt tospovirus* (TSWV). When we released a variety resistant to one or even two, it often was badly susceptible to another. We needed to select for resistance to all four simultaneously.

Each year, we cross agronomically superior parents with sources of high-level resistance that are often not agronomically desirable in the Virginia-Carolina area, including runner-type sources. After the cross, we use a form of “shuttle” breeding to get to genetically stable inbred lines in three years. Starting the second generation after the cross, we make plant selections for pod and seed characteristics in North Carolina. We use a winter seed nursery in Puerto Rico to grow a second generation each year, then test the selected families for resistance to the four diseases in separate trials, identifying the best families overall and making plant selections within those families. By the sixth generation after the cross, the families are genetically stable breeding lines that have improved disease resistance and then proceed through the multi-year multi-location testing program that eventually leads to variety release. Highly resistant lines that do not measure up agronomically may be recycled as parents in the crossing program.

Two varieties have been released from this project: ‘Bailey’ in 2008 and ‘Sugg’ in 2009. Certified seed of Bailey was available to growers for the 2011 growing season, as were limited amounts of Sugg. Both have partial resistance to all four diseases and also have excellent yield potential. At federal support prices for peanuts, saving one or two applications of leaf spot fungicide or a single application of Sclerotinia preventives could mean the difference between profit and loss. Resistant cultivars will help to maintain peanut production and the peanut seed market in North Carolina. In recent years, we have incorporated greater levels of disease resistance and have advanced only families carrying the high-oleic seed oil gene in our accelerated selection program.