PINEMAP Year 5 Progress Report 2
December 2015
Aim 3 (Genetics)

This is the second Aim progress report for year 5 (covering activity from July 1, 2015-November 30, 2015). The information provided in these reports is used to track Aim-level outputs and outcomes over the course of the project and to fulfill NIFA reporting requirements.

The purpose of this report is to gather information on progress since the previous progress report in July 2015.

To streamline this process, information reported in July 2015is provided below, so you will simply need to review and update each section as necessary.

Please return the completed report to Grace no later than December 1st.

PROGRESS SUMMARY:

Please provide 1-3 paragraph summary of key areas of progress since June 30, 2015. You might consider writing this section after completing the remaining sections.

Key areas of progress since June 30, 2015 are SNP discovery and association with phenotypic traits relevant to PINEMAP objectives, including both productivity and wood quality traits with commercial impacts as well as physiological traits relevant to plantation resilience goals.

Additional phenotypic data on freezing tolerance have also been collected on the ADEPT2 association population; these data will be used in testing for associations between SNP genetic variants and freezing tolerance. Cost-effective, high-throughput methods for genotyping SNP variants in specific genes are under development to facilitate routine application of genetic marker information in applied breeding programs.

Aim 3 personnel contributed to beta-testing of the web-based DSS tool introduced to the PINEMAP research team at the Dec 4 2015 All-Team PINEMAP web meeting, in addition to earlier contributions to design and implementation of the DSS.

OUTCOMES/IMPACTS

Outcomes and ***Impacts*** are tangible results for stakeholders and society that the project has produced to advance on the societal challenge (e.g., ***changes in knowledge, actions, or conditions*** that result from project activities). Outcomes and impacts are similar, but impacts are typically longer-term; outcomes are used as a nearer-term proxy for impacts.

Describe how Aim-level activities, results, findings, techniques, or products contribute to project-level outcomes and impacts (e.g., changes in knowledge, actions, or conditions resulting from activities).

A narrative has been drafted below. Please modify or update as necessary.

Aim 3 activities will contribute to project-level outcomes and impacts by providing a genetic deployment tool necessary for stakeholders to make sound genetic deployment decisions under changing climatic conditions and by discovering genes and markers associated with important mitigation and adaptation traits that can be incorporated into future breeding programs. Aim 3 will work closely with Aims 1 and 2 to provide input and benefit from insights gleaned from the stand-level studies and modeling to make tree improvement more efficient. Simultaneously, knowledge of traits varying at the gene level should contribute to the ecophysiology studies. Integration of the genetic data collected on pine populations during PINEMAP research with the draft genome sequence assembly of loblolly pine produced by the PineRefSeq project will continue to be an important aspect of Aim 3 efforts, because this integration both adds value to the genome assembly and leverages the power of the genome sequencing project for applied breeding efforts. This integration effort will be one focus of Aim 3 activities during the requested no-cost extension; it was not part of the original proposal objectives because the PineRefSeq project was funded in the same funding cycle as the PINEMAP project. A second focus of Aim 3 activities will be continued refinement of the web-based Decision Support System, which will integrate the seed deployment guidelines developed from Aim 3 research with results from other Aims to inform stakeholders about climate change effects on pine plantations.

OUTPUTS

Products

Products include published or in press peer-reviewed publications; other written materials such as white papers, research summaries, fact sheets, or popular press articles; audio or video products; etc.

The lists below summarize products reported in the July 2015Progress Report (March 1, 2015-June 30, 2015)
Please update as necessary (including in press publications that are now published) and highlight in yellow any new products added to the list for the December 2015 Progress Report.

Peer-reviewed publications

Dasgupta, M. G., V. Dharanishanthi, I. Agarwal, and K. V. Krutovsky, 2015 Development of genetic markers in *Eucalyptus* species by target enrichment and exome sequencing. *PLoS One* **10**(1): e0116528. doi:10.1371/journal.pone.011652.

Egbäck, S., B.P. Bullock, F. Isik, and S. McKeand. 2015. Height-diameter relationships for different genetic planting stock of loblolly pine at age six. For. Sci. 61(3): 424-428.

Farjat, A.E., Isik, F., Reich, B.J., Whetten, R.W., McKeand, S.E. 2015. Modeling Climate Change Effects on the Height Growth of Loblolly Pine. Forest Science 61 (4, 5): 703-715.

Holliday JA, Zhou L, Bawa R, Zhang M, Oubida RW (2015) Evidence for extensive parallelism but divergent genomic architecture of adaptation along altitudinal and latitudinal gradients in *Populus trichocarpa*. New Phytologist (Online Early). DOI: 10.1111/nph.13643

Kim, T.J., B.P. Bullock, and S.E. McKeand. 2015. Spatial autocorrelation among different levels of genetic control and spacings in loblolly pine. For. Sci. 61(3): 438-444.

Koralewski, T. E., J. E. Brooks, and K. V. Krutovsky, 2014 Molecular evolution of drought tolerance and wood strength related candidate genes in loblolly pine (*Pinus taeda* L.). *Silvae Genetica* **63**(1-2): 59-66.

Koralewski, T. E., H.-H. Wang, W. E. Grant, and T. D. Byram. 2015. Plants on the move: Assisted migration of forest trees in the face of climate change. *Forest Ecology and Management* 344: 30-37.

Krutovsky, K. V., 2014 Prospects for genomic research in forestry. *Siberian Journal of Forest Science* **1**(4): 11-15.

Krutovsky, K. V., N. V. Oreshkova, Yu. A. Putintseva, A. A. Ibe, K. O. Deich, and E. A. Shilkina, 2014 Preliminary results of *de novo* whole genome sequencing of Siberian larch (*Larix sibirica* Ledeb.) and Siberian stone pine (*Pinus sibirica* Du Tour.). *Siberian Journal of Forest Science* **1**(4): 79-83.

Krutovsky, K. V., I. N. Tretyakova, N. V. Oreshkova, M. E. Pak, O. V. Kvitko, and E. A. Vaganov, 2014 Somaclonal variation of haploid in vitro tissue culture obtained from Siberian larch (*Larix sibirica* Ledeb.) megagametophytes for whole genome *de novo* sequencing. *In Vitro Cellular and Developmental Biology – Plant* **50**(5): 655-664.

Smith, B.C., B.P. Bullock, F. Isik, and S.E. McKeand. 2014. Modeling genetic effects on growth of diverse provenances and families of loblolly pine across optimum and deficient nutrient regimes. Can. J. For. Res. 44: 1453–1461.

Sadovsky, M. G., V. V. Birukov, Y. A. Putintseva, N. V. Oreshkova, E. A. Vaganov, and K. V. Krutovsky, 2015 Symmetry of Siberian Larch Transcriptome. *Journal of Siberian Federal University. Biology* **8**(3): 278-286.

Sadovsky, M. G., E. I. Bondar, Y. A. Putintseva, N. V. Oreshkova, E. A. Vaganov, and K. V. Krutovsky, 2015 Seven-Cluster Structure of Chloroplast Genome. *Journal of Siberian Federal University. Biology* **8**(3): 268-277.

Semerikov, V.L., Y.A. Putintseva, N.V. Oreshkova, and K.V. Krutovsky, 2015 Development of mitochondrial DNA markers in Scots pine (*Pinus sylvestris* L.) for population genetics and phylogeography studies. *Russian Journal of Genetics* **51**(12): 1386-1390.

Shilkina, E. A., N. V. Oreshkova, A. A. Ibe, K. O. Deich, and K. V. Krutovsky, 2014 Development of cytoplasmatic SSR-markers in *Pinus sibirica* Du Tour for population genetic studies. *Siberian Journal of Forest Science* **1**(4): 21-24.

Westbrook J. W., V. E. Chhatre, L-S. Wu, S. Chamala, L. G. Neves, P. Muñoz, P. J. Martínez-García, D. B. Neale, M. Kirst, K. Mockaitis, C. D. Nelson, G. F. Peter, J. M. Davis, and C. S. Echt. 2015. A consensus genetic map for Pinus taeda and Pinus elliottii and extent of linkage disequilibrium in two genotype-phenotype discovery populations of P. taeda.
G3 (Bethesda), in press (available online at
http://www.g3journal.org/content/early/2015/06/11/g3.115.019588.abstract)

Yeaman S, Hodgins KA, Suren H, Nurkowski KA, Rieseberg LH, Holliday, JA, Aitken SN (2014) Conservation and divergence of gene expression plasticity following ~140 million years of evolution in lodgepole pine (*Pinus contorta*) and interior spruce (natural hybrid populations of *Picea glauca* and *Picea engelmannii*). *New Phytologist*, 203(2):578-91.

Zapata-Valenzuela, J., F. Ogut, A. Kegley, W.P. Cumbie, F. Isik, B. Li, and S.E. McKeand. 2014. Seedling evaluation of Atlantic Coastal and Piedmont sources of Pinus taeda L. and their hybrids for cold hardiness. For. Sci. 61(1):169–175.

Zhang, C., R. Finkeldey, and **K. V. Krutovsky**, 2015 Genetic diversity and parentage analysis of aspen demes. *New Forests* (http://link.springer.com/article/10.1007/s11056-015-9501-9)

Theses/Dissertations

Identifying genetic variation in loblolly pine. Laura A. Townsend, MS thesis. Available at http://www.lib.ncsu.edu/resolver/1840.16/10731

Other publications

None reported in July 2015 report.

Audio/video products

None reported in July 2015 report.

Events/Activities

Events/activities include presentations (oral and poster) given at meetings or conferences; workshops/trainings/courses conducted; and experiments/surveys/data collection conducted.

The table(s) below summarize products reported in July 2015Progress Report (March 1, 2015-June 30, 2015)

Please update as necessary and highlight in yellow any new products added to the list for the December 2015 Progress Report.

Presentations

Author(s)/Presenter(s)	Title	Type	Date	Venue/Location
Belokon, M. M., T. A. Polyakova, A. V. Shatokhina, E. A. Mudrik, Yu. S. Belokon, Yu. A. Putintseva, N. V. Oreshkova, D. V. Politov, and K. V. Krutovsky	Developing nuclear microsatellite markers in Siberian stone pine (*Pinus sibirica* Du Tour) using whole genome sequencing data.	Oral Presentation	August 25-31, 2015	4-th International conference “Conservation of forest genetic resources in Siberia”
Bondar, E. I., O. A. Ushakova, Yu. A. Putintseva, N. V. Oreshkova, and K. V. Krutovsky	Assembly and annotation of Siberian larch (*Larix sibirica* Ledeb.) chloroplast genome and the search for polymorphic genetic markers (SSRs and SNPs).	Oral Presentation	June 17–21, 2015	The 3rd International Conference “Plant Genetics, Genomics, Bioinformatics and Biotechnology”, Novosibirsk, Russia
Bondar, E. I., Yu. A. Putintseva, N. V. Oreshkova, and K. V. Krutovsky	Study of Siberian larch (*Larix sibirica* Ledeb.) chloroplast genome and development of polymorphic chloroplast markers.	Oral Presentation	August 25-31, 2015	4-th International conference “Conservation of forest genetic resources in Siberia”
Casola, C.	Genomics research at Texas A&M	Presentation (Meeting)	May 19-20, 2015	WGFTIP Contact Representatives Meeting, College Station, TX
Cuervo, L., M. Arend, M. Müller, R. Finkeldey, and K. Krutovsky	Genetic analysis of European beech populations across a precipitation gradient using microsatellite markers and SNPs in candidate genes.	Oral Presentation	August 24-28, 2015	Integrated AdapCAR and EVOLTREE Conference and summer school on ‘Global change and the evolutionary potential of forest trees’, Selfoss, Iceland.
Farjat, A., B. Reich, F. Isik, R. W. Whetten, S. E. McKeand	Bayesian spatial modeling for loblolly pine seed source movements	Poster Presentation	June 3 – 4, 2015	PINEMAP Annual Meeting (presented by Ross Whetten)
Festa, A. R. and R. W. Whetten	An alternative approach to selection within families	Poster presentation	June 3 – 4 and June 9 – 10, 2015	PINEMAP Annual Meeting, Athens GA, and Southern Forest Tree Improvement Conference, Hot Springs AR
Holliday JA, Suren H	Association mapping of climate-related traits in spruce and pine	Presentation (meeting)	June 9, 2014	Forest Genomics Symposium, Vancouver, BC, Canada

Author(s)/Presenter(s)	Title	Type	Date	Venue/Location
Johnson, J. S., D. M. Cairns, K. D. Gaddis, and K. V. Krutovsky, 2015	Unraveling Gene Flow and Genome Variation in Three Forest Species Using a Landscape Genomics Approach.	Oral Presentation	July 24, 2015	Landcare Research Seminar Series, Lincoln, NZ.
Johnson, J. S., D. M. Cairns, K. D. Gaddis, K. V. Krutovsky, and K. Konganti	Genomics of Long-Distance Dispersal in Mountain Hemlock.	Oral Presentation	April 21-25, 2015	Association of American Geographers Annual Conference, Chicago, IL.
Johnson, J. S., K. Konganti, D. M. Cairns, K. D. Gaddis, and K. V. Krutovsky, 2015 Winner: Best Paper Presentation.	Mountain Hemlock Genomics: A ddRADseq Approach.	Oral Presentation	March 6, 2015	The Second Annual Geography Graduate Student Research Symposium, Texas A&M University, College Station, TX.
Koralewski, T.E., H.-H. Wang, W.E. Grant, and T.D. Byram	Mitigating climate change effects on plants through assisted migration with the application of a modeling approach: A lesson from forest trees.	Poster Presentation	August 9-14, 2015	100th Ecological Society of America Annual Meeting, Baltimore, MD
Koralewski T.E., T.D. Byram, H.-H. Wang, W.E. Grant	Modeling responses of forest trees to changing climate based on historical provenance trial data	Presentation (Meeting)	November 12, 2015	Camcore 2015 Annual Meeting, College Station, TX
Koralewski, T.E., T.D. Byram, H.-H. Wang and W.E. Grant	Deployment and procurement of loblolly pine (*Pinus taeda* L.) seed sources guided by the application of categorical universal response function (CURF)	Presentation (Meeting and extended abstract)	June 9-10, 2015	Southern Forest Tree Improvement Conference, Hot Springs, AR
Koralewski, TE, M Mateos, and KV Krutovsky	Phylogeny of major southern pines (subsection *Australes*, genus *Pinus*, family Pinaceae)	Poster Presentation	June 9-10, 2015	Southern Forest Tree Improvement Conference, Hot Springs, AR
Koralewski, T.E., T.D. Byram, H.-H. Wang and W.E. Grant	Supporting loblolly pine deployment and procurement decisions: A modeling approach	Poster Presentation	June 3-4, 2015	PINEMAP Annual Meeting Athens, GA
Koralewski, T.E.	Where from and where to: Using climate models to guide deployment	Presentation (Meeting)	May 19-20, 2015	WGFTIP Contact Representatives Meeting College Station, TX
Krutovsky, K. V.	Genomic and epigenomic mechanisms of adaptation in the forest tree species.	Oral Presentation	August 25-31, 2015	4-th International conference "Conservation of forest genetic resources in Siberia"
Krutovsky, K., V. Chhatre, M. Lu, T. Byram, J. Wegrzyn, D. Neale, C. Loopstra	Population and landscape genomics to study local adaptation in loblolly pine (*Pinus taeda*) populations and to breed more climate change resilient trees.	Oral Presentation	October 5–11, 2014	XXIV IUFRO World Congress, Salt Lake City, USA

Author(s)/Presenter(s)	Title	Type	Date	Venue/Location
Krutovsky, K. V.	Genome study of Siberian stone pine (*Pinus sibirica*) and Siberian larch (*Larix sibirica*).	Oral Presentation	June 26-28, 2014	Third meeting of the Alpine Forest Genomics Network (AForGeN), Fafleralp, Lötschental (Canton of Valais), Switzerland
Krutovsky, K. V., V. E. Chhatre, M. Lu, T. D. Byram, J. L. Wegrzyn, D. B. Neale, C. Loopstra	High-Throughput Genome-Wide Genotyping, Targeted Sequencing and Association Mapping of Adaptive and Breeding Traits in Loblolly Pine (*Pinus taeda* L.) Populations.	Oral Presentation	January 11-15, 2014	*Plant & Animal Genome XXII*. The International Conference on the Status of Plant and Animal Genome Research, San Diego, CA
Krutovsky, K. V., N. V. Oreshkova, Yu. A. Putintseva, D. A. Kuzmin, V. V. Sharov, V. V. Biryukov, S. V. Makolov, K. O. Deych, E. I. Bondar, O. A. Ushakova, and E. A. Shilkina	*De novo* sequencing of conifer megagenomes.	Oral Presentation	June 17–21, 2015	The 3rd International Conference “Plant Genetics, Genomics, Bioinformatics and Biotechnology”, Novosibirsk, Russia
Krutovsky, K. V., Oreshkova N.V., Putintseva Yu.A., Kuzmin D.A., Pavlov I.N., Sharov V.V., Biryukov V.V., Makolov S.V., Deych K.O., Bondar E.I., Ushakova O.A., Ibe A.A., Shilkina E.A., Sadovsky M.Yu., Vaganov E.A.	*Pinus sibirica* and *Larix sibirica* whole genome de novo sequencing.	Oral Presentation (Invited speaker)	November 30th - December 4th 2015	The ProCoGen Final Open International Conference and Workshops on Promoting Conifer Genomic Resources, Orléans, France
Krutovsky, K. V., I. Chubugina, A. Razdaivodin, A. Radin, D. Romashkin	Study of genetic mutations in Scots pine (*Pinus sylvestris* L.) populations growing under the chronic radioactive contamination	Oral Presentation (Invited speaker)	November 23-26, 2015	International conference on "Plant Responses to Anthropomorphic Heavy Metal and Radioactive Pollution", College of Resources and Environment, Shanxi Agricultural University, Taigu, Shanxi, China.
K. V. Krutovsky, N. V. Oreshkova, E. A. Babushkina, L. V. Belokopytova, T. V. Kostyakova, A. M. Grachev, and E. A. Vaganov	Preliminary results on using methods of dendrochronology and genome wide genotyping for studying the genetic mechanisms of homeostasis, heterosis and genetic resistance towards the environmental factors in Siberian larch.	Oral Presentation	January 20-21, 2015	The International Dendroecological Seminar, Krasnoyarsk, Russia.
Loopstra, C.	What can you do with a million molecular markers?	Presentation (Meeting)	May 19-20, 2015	WGFTIP Contact Representatives Meeting, College Station, TX
Lu,M., K. Krutovsky, C.D. Nelson, T. Koralewsky, T. Byram, C. Loopstra	Exome capture in a population of *Pinus taeda* L.	Poster Presentation	June 3-4, 2015	PINEMAP Annual Meeting, Athens, GA

Author(s)/Presenter(s)	Title	Type	Date	Venue/Location
Lu, M., K. Krutovsky, C.D. Nelson, T. Koralewski, T. Byram, C. Loopstra	Exome genotyping and association genetics of environmental adaptation and stress mitigation traits in a clonally tested loblolly pine (*Pinus taeda* L.) population.	Poster Presentation	December 3, 2015	Molecular & Environmental Plant Science Student Association and Horticulture Graduate Council Annual Fall Student Poster Symposium, College Station, TX
Oreshkova, N. V., Yu. A. Putintseva, D. A. Kuzmin, V. V. Sharov, V. V. Biryukov, S. V. Makolov, K. O. Deich, A. A. Ibe, E. A. Shilkina, and K. V. Krutovsky,	Genome sequencing and assembly of Siberian larch (*Larix sibirica* ledeb.) and Siberian pine (*Pinus sibirica* Du Tour) and prelimenary transcriptome data.	Oral Presentation	August 25-31, 2015	4-th International conference “Conservation of forest genetic resources in Siberia”
Oreshkova, N. V., Yu. A. Putintseva, D. A. Kuzmin, V. V. Sharov, V. V. Biryukov, S. V. Makolov, K. O. Deych, A. A. Ibe, E. A. Shilkina, and K. V. Krutovsky	The whole de novo genome sequencing and assembly of Siberian larch (*Larix sibirica* Ledeb.) and Siberian pine (*Pinus sibirica* Du Tour.).	Oral Presentation	June 17–21, 2015	The 3rd International Conference “Plant Genetics, Genomics, Bioinformatics and Biotechnology”, Novosibirsk, Russia
Putintseva, Yu. A., V. V. Sharov, D. A. Kuzmin, S. V. Makolov, N. V. Oreshkova, and K. V. Krutovsky	Challenges of assembling huge conifer genomes.	Oral Presentation	June 17–21, 2015	The 3rd International Conference “Plant Genetics, Genomics, Bioinformatics and Biotechnology”, Novosibirsk, Russia
Semerikov, V. L., Yu. A. Putintseva, N. V. Oreshkova, and K. V. Krutovsky,	Development of mitochondrial DNA markers in key Siberian boreal forest conifer species based on genome sequencing and their use in phylogeography.	Oral Presentation	August 25-31, 2015	4-th International conference “Conservation of forest genetic resources in Siberia”
Westbrook JW, Chhatre VE, Wu LS, Chamala S, Neves LG, Muñoz P, Martínez-García PJ, Neale DB, Kirst M, Mockaitis K, Nelson CD, Peter GF, Davis JM, Echt CS	An annotated consensus genetic map for Pinus taeda L. and extent of linkage disequilibrium in three genotype-phenotype discovery populations	Poster Presentation	June 3-4, 2015 presented by Echt; and June 8-9, 2015 presented by Nelson	PINEMAP Annual Meeting, Athens, GA; and Southern Forest Tree Improvement Conference, Hot Springs, AR
Whetten, Ross	What are genomic technologies, and how can they be applied to tree breeding?	Invited oral presentation	June 23-24, 2015	Joint meeting of the Western Forest Genetics Association and the Northwest Seed Orchard Management Association, Seattle, WA.

Trainings, workshops, and courses

Krutovsky, K. V., 2014. Sequencing and conservation genomics. ProCoGen Dissemination Workshop “Genomics and the conservation of conifer genetic resources”, September 1-3, 2014, Kámoni Arboretum Szombathely, Hungary. (http://www.procogen.eu)

Krutovsky, K.V., 2014. Importance of genetic studies for understanding of structure, function and adaptation of the forest ecosystems and for Siberian boreal forest management. The Training Workshop for Young Scientists “Problems and prospects of the forest ecosystem studies”, September 15-19, 2014, Krasnoyarsk, Russia. (http://forest.akadem.ru/Konf/2014/IF/Program_school.pdf)

Experiments, surveys, and data collection

None reported in July 2015.

PROGRESS NARRATIVE

Provide a brief summary of progress on each deliverable/task/input/output listed below. Please provide a *brief* summary of progress on each deliverable/task/input/output listed below. In many cases, a one sentence summary may suffice. If there is no progress update on an item, leave blank. Please do not include any figures or tables, but please do include quantifiable measurements, if available (i.e., # of plots measured, # of samples, # of runs, # of people reached, etc.)

Text below summarizes products reported in the July 2015Progress Report (March 1, 2015-June 30, 2015). Older entries are grey

Please update as necessary and highlight in yellow any new products added to the list for the July 2015 Progress Report, with approximate month of estimated completion.

Genotyping

Task: Genotyping complete (sequencing done, data analysis on going) (5/31/15)

Sequencing data collection is largely complete for the groups at Texas, Florida, and Virginia Tech; the group at NC State will continue sequencing through the end of the project to maximize the number of different parents for which seedling gene expression measurements are available. These seedling gene expression measurements will then be utilized during the no-cost extension to expand the scope of the seed deployment guidelines. Additional sequencing experiments will also be undertaken at NC State to map regions of the pine genome that may be involved in gene regulation, as such data will add significant value to the PineRefSeq draft genome assembly and to future efforts to use molecular genetic markers in the context of applied breeding programs. Experiments are underway at NC State to test multiplex PCR amplification and high-throughput DNA sequencing methods for targeted genotyping of specific SNPs as a cost-effective means of using genetic marker – trait associations in practical tree breeding programs. Coordination of methods among the three university-based cooperative breeding programs and their respective cooperators is a goal, so that a common set of methods and resources will be available for use by both public- and private-sector breeding programs in future applications of PINEMAP research findings to applied breeding.

Sequencing and genotyping have been completed at Texas A&M.

Deliverable: Upload ADEPT2 phenotypes into TerraC (8/31/15)

No progress reported in July 2015 report. All the measurements have been completed and data is ready for uploading.

Task: Discover genetic variant data (SNPs in VCF files) (8/31/15)

No progress reported in July 2015 report. VCF files are ready for sharing at TAMU.
A total of 972,720 SNPs were detected by TAMU researchers after filtering and analysis of exome capture sequence data from the ADEPT2 population. Analysis of these data shows that linkage disequilibrium decays in the loblolly pine genome over shorter distances than was previously reported. Population structure analysis using unlinked SNPs demonstrated two distinct clusters representing west and east parts of the loblolly pine range, which corresponds to

the Mississippi River discontinuity in the native range of loblolly pine. After correction for multiple testing, a total of 45 SNPs were associated with the traits of mean branch angle (n=3), carbon isotope discrimination (n=4), diameter (n=8), height (n=9), nitrogen content (n=7), and width (n=14). Associations with more traits are under way.

Deliverable: Upload genetic variant data into TreeGenes (11/30/15)
No progress reported in July 2015 report. Data is available for uploading at TAMU.

Deliverable: Tree breeding tools available to breeding programs (2/29/16)
No progress reported in July 2015 report.
NC State researchers have obtained sequence data for over 100 target regions from 960 individuals from a progeny test population in an initial trial of the multiplex-PCR-based genotyping system. Methods to detect SNPs within these regions and recover linkage phase information for SNP haplotypes are under development, to maximize the information content available from the SNPs detected in each amplified target region.

Deliverable: Better adapted germplasm (2/29/16)
No progress reported in July 2015 report.

Seed deployment tool for DSS

Deliverable: Version 1 Seedling deployment tool (2/28/15)
North Carolina State University: Additional analysis of variation among families within seed sources, using maximum and minimum annual temperature and total precipitation as climate variables, shows that 40% to 60% of the variation in family performance relative to locally-adapted checklot families can be explained by three climate factors. The predictive power of these models will be tested by cross-validation to evaluate the stability of the models, and additional climate factors will be tested to assess which models have the best predictive power. If this method is successful with the PSSSS data, it can be extended to include other progeny test data to evaluate the approach across a more diverse set of germplasm from the current breeding population.
The positive preliminary results obtained from analysis of seedling gene expression analysis results in conjunction with field trial data suggests that it may be possible to develop methods to predict which families within a specific provenance or seed source will show above-average resilience to variation in environmental conditions. This possibility will be explored in further experiments with germplasm for which both seed samples and field test data are available.

Texas A&M University has used a multinomial logit regression approach to develop a Categorical Universal Response Function (CURF) to delineate potential deployment zones for loblolly pine (*Pinus taeda* L.) using 15 year measurements from the Western Gulf Forest Tree Improvement Program (WGFTIP) Geographic Seed Source Study (GSSS). The CURF uses performance categories for the response variable, and the model assigns a probability score of a given seed source's response falling into each of these categories. Minimum temperature of the coldest month, summer precipitation, and variation measures of these two metrics for both the seed source provenance and the test location were used as independent variables. Planted tree volume, accounting for both survival and growth (a proxy for timber yield) was used as the response variable. Even with this limited number of environmental input variables, model performance was good, with the AUC score ranging from 0.785 to 0.808, depending on the parameters included and the evaluation criteria. The models developed with the CURF approach can be readily implemented in a Decision Support System as they 1) suggest sets of adapted families from which foresters can choose based on local knowledge, 2) can be easily expanded to include other variables, and 3) can be applied to outputs from projected climate scenarios to extrapolate into the future.

NC State University researchers have continued to explore refinements in statistical modeling approaches to build predictive models suitable for incorporation into the web-based DSS. Recent efforts have focused on Bayesian non-parametric methods that are more flexible than the multiple regression approach used in the first round of analyses. The Bayesian method provides a description of the uncertainty of outcomes as well as the expected outcome, which would allow land owners and managers to incorporate those uncertainties into their own risk-benefit analysis to determine the most favorable course of action given their own financial situations.

Input: Beta testing Version 1 of seedling deployment tool (5/31/15)

Feedback was provided to the Decision Support System development team during Q1Y5 regarding the prototype DSS website, and ongoing conversations with the DSS developers and outside stakeholders are ongoing to continue to maximize the value of the DSS to stakeholders.

Deliverable: Final seedling deployment tool (8/31/15)

The AIM 3 Group will be working with the Deployment Decision Support Group to produce an interactive web application to produce ‘heat-maps’ showing historic and projected values for climate variables identified as important in our analysis

The DSS tool was unveiled at the All-Team PINEMAP meeting on Dec 4, 2015.

New markers for genotypes in ADEPT2, CCLONES, and PSSSS populations (Goal Date in the proposal was in year 2 to 4, say Q1Y5)

Virginia Tech is using GBS to genotype ~1000 progeny of the PSSSS trial at Appomattox, VA, which will complement ongoing work at NC State that involves genotyping the same families at more southern sites. This will allow for testing of predictions based on genome-wide data across two different points in the climate space in which loblolly pine is planted. We used double-digest GBS (PstI/MspI) and sequenced 96 samples per lane. Following QC (removing adapter sequence, demultiplexing, etc) we retained 5.04 million reads per sample on average with a standard deviation of 1.1 million. Only ~10 samples failed. Once bioinformatics tasks are complete, these data will be coupled with growth data available from NC State (co-PI McKeand) to conduct marker-trait association mapping as well as genomic selection. Availability of data on associations of specific genes or genomic regions with height growth in the same families grown in contrasting sites (Appomattox County Virginia versus Screven County, Georgia) will allow tests of the hypothesis that different genes are involved in adaptation to different environmental conditions. Following filtering on quality and missing data, 60,902 SNP markers were identified. These data are being coupled with growth data provided by NC State (co-PI McKeand) to conduct marker-trait association mapping as well as genomic selection. Preliminary GWAS analysis reveals numerous associations with height, diameter, straightness, as well as rust resistance.

Phenotypes for adaptive traits in ADEPT2 & CCLONES (Goal Date?)

In collaboration with Dana Nelson (USFS), Virginia Tech researchers are measuring cold hardiness for the ADEPT2 population at Saucier, MS. We will begin with a preliminary test in late fall, and subsequently completed the full test in January 2015. Results suggest substantial variance in cold hardiness that corresponds to latitude of origin, and these data are being used to identify the underlying genes through GWAS with data generated by the group at TAMU. ~~and assuming genetic variation in this trait exists, we will proceed to a full replicated test in winter.~~

Phenotypes growth and responsiveness to nutrition in ADEPT2 & CCLONES (Goal Date?)

No progress reported in July 2015.

Phenotype wood density and lignin content of ADEPT2) (Goal Date?)
No progress reported in July 2015. Gary Peter (UF) has phenotyped the ADEPT2 population for wood density and chemistry. .

Discover alleles associated with water relations (existing phenotypes, new markers) (Goal Date?)
No progress reported in July 2015. Four SNPS with positive associations to carbon isotope values (an estimate of water use efficiency) were discovered in the ADEPT2 population.

Discover alleles associated with nitrogen responsiveness (existing phenotypes, new markers) (Goal Date?)
No progress reported in July 2015.

Discover & validate alleles and genes associated with growth and adaptive traits in 3 populations (new markers) (Goal Date?)
No progress reported in July 2015. SNPs associated with of mean branch angle (n=3), diameter (n=8), height (n=9), nitrogen content (n=7), and crown width (n=14) were discovered in the ADEPT2 population.

Molecular tools for accelerating tree improvement and deployment (Goal Date in the proposal is the end of the project, Q4Y5)
No progress reported in July 2015. 972,720 SNPs were discovered in the ADEPT2 population after filtering for those found in regions sequenced at least 10 times in 90% of the population.

Develop new region-wide cross population for southwide field test (Goal Date?)
No progress reported in July 2015.

BROAD IMPACTS

Provide a short narrative describing broad impacts (i.e., far-reaching and possibly unanticipated outcomes resulting from Aim work). Specifically, please highlight leveraged funds and/or partnerships with other projects/external collaborations.

The NCSU Cooperative Tree Improvement Program is also participating in another NIFA Coordinated Agricultural Project, the Integrated Biomass Supply System (IBSS) project funded at University of Tennessee-Knoxville under the bioenergy component of the CAP program. The IBSS research conducted by NCSU CTIP is focused on chemical and physical properties of loblolly pine biomass, with a strong focus on wood. These properties are relevant to PINEMAP objectives because they affect the market for southern pine forest products, including solid wood products, pulp and paper as well as potential bioenergy markets that may develop.

Close ties are maintained between PINEMAP researchers and the PineRefSeq CAP, also funded by the bioenergy component of the CAP program, to fully exploit the value of the draft loblolly pine genome sequence for the breeding and genetic analysis objectives of the IBSS and PINEMAP research efforts. Texas A&M University applied the results of the draft loblolly pine reference genome assembly and annotation from PineRefSeq team for designing the capture probes used in the Nimblegen hybrid-capture sequencing experiment now being analyzed. The SNPs discovered from the captured exome sequences will be a supplement for the current pool of molecular markers which could be used in screening for better-adapted genotypes.

The application of the multinomial logit regression approach to the development of environmental models to guide assisted migration appears to have broader application to other areas such as the ecophysiological modeling of invasive plant movement.

TRAINING

A CUMULATIVE list of all Aim 1 undergraduate and graduate students, postdocs, and technical/research personnel trained under this project and descriptions of their research focus and/or role in the project is provided below. Additions/ changes made in the July 2015 progress report are highlighted in blue. Please update as necessary and highlight in yellow any updates made for this progress report.

Last name	First name	Position	University	Role
Bawa	Rajesh	Ph.D. Student	VT	Research focus: Conducting Phenotyping of adaptive traits (i.e., cold hardiness and bud phenology) in the loblolly pine progeny trials and working with universities across region to conduct genome-wide association mapping between these traits and the re-sequencing data.
Chhatre	Vikram	Postdoc	USFS-SRS	Construction of a consensus linkage map in Pinus taeda and P. elliottii.
Farjat	Alfredo	Ph.D. Student	NCSU	Research focus: Carrying out joint analysis of progeny test data and climate data for the Cooperative Tree Improvement Program.
Festa	Adam	Ph.D Student	NCSU	Research focus: Using data on variation both in gene expression and in coding-sequence variation to model genetic control of phenotypic variation in loblolly pine breeding populations
Koralewski	Tomasz	Postdoc	TAMU	Building a location database for the progeny tests and the parents represented in those progeny tests that can be linked to climatic data, and performing URF analysis to optimize seedling deployment.
Lu	Mengmeng	Ph.D. Student	TAMU	Research focus: Evaluated exome capture systems and used the Nimblegen system to genotype 375 trees from the ADEPT2 population using HiSeq high-throughput sequencing. Probes were designed for 199,723 exons. Currently using the discovered SNPs for genome and association analyses.
Kohlway	Will	Undergraduate Research Assistant	NCSU	Working on pilot scale experiments to test genotyping-by-sequencing as a cost-effective means of genotyping the experimental populations. (Deleted in Sept 2014 report, but this is cumulative list)
Rusche	Ben	Undergraduate Research Assistant	NCSU	Working on pilot scale experiments to test genotyping-by-sequencing as a cost-effective means of genotyping the experimental populations. (Deleted in Sept 2014 report, but this is cumulative list)
Townsend	Laura	MS	NCSU	Research focus: Genetic variation in loblolly pine for adaptability to climate variables.
Zhang	Jianxing	Ph.D. Student	UF	Research focus: conducting analyses linking progeny test performance with climatic data and developing a database for the URF analysis to optimize seedling deployment.