

Starptautiska zinātniska konference "Adaptīvo pazīmju ģenētiskā variācija" (Genetic variations of adaptive traits)

No 2011. gada 30. novembra līdz 1. decembrim LVMI "Silava" organizēja starptautisku zinātnisko konferenci "Adaptīvo pazīmju ģenētiskā variācija" (Genetic variation of adaptive traits).

Konferences darbā iesaistījās 24 dalībnieki no 3 Baltijas valstīm, pārstāvot 5 zinātniskās institūcijas. Tika prezentēti galvenokārt ESF finansēta pētījuma "Ģenētisko faktoru nozīme adaptētās spējīgu un pēc koksnes īpašībām kvalitatīvu mežaudžu izveidē" 2010. un 2011. gada iegūto rezultātu apkopojums, ietverto šādus jautājumu blokus:

- koku radniecība audzes ietvaros, tās noteikšanas metodes un potenciālā ietekme uz adaptāciju;
- dažādu sugu koku augstuma pieauguma veidošanās sezonālā dinamika, saikne ar meteoroloģiskajiem faktoriem un atšķirības starp dažādu genotipu kokiem;
- introducētas koku sugas (Klinškalna priede) produktivitāte un to ietekmējošie faktori Baltijas valstīs;
- ģenētisko faktoru un atjaunošanas/kopšanas ietekme uz jaunaudžu parametriem;
- koksnes īpašības un to ģenētiskā nosacītība dažādu sugu kokiem;
- koku noturība pret biotisko un abiotisko faktoru ietekmi, genotipu atšķirības un molekulārās ģenētikas metožu pielietošana šo atšķirību cēloņu analizē prognozēšanā;
- apšu hibrīdu pazemes biomasas un klonu atšķirības šajā aspektā.

Detalizēta konferences darba kārtība (angļu val.) pievienota relīzes pielikumā.

Iegūtie rezultāti liecina, ka koku ģenētikai ir būtiska loma, nosakot to augšanas sezonālos procesus, atbildes reakciju uz meteoroloģiskajiem apstākļiem, kā arī noturību pret dažādiem nelabvēlīgiem vides faktoriem un koksnes īpašības. Tas jāņem vērā, plānojot meža atjaunošanu sagaidāmo klimata izmaiņu kontekstā.

Konferences prezentācijas (angļu valodā, pievienotas relīzes pielikumā):

- Zeps M. Annual shoot development dynamics of hybrid aspen: results of 2010, 2011;
- Jansons Ā. Annual shoot development dynamics of Scots pine (genetic differences);
- Lībiete-Zālīte Z. Climate impacts on lodgepole pine (*Pinus contorta* var. *latifolia*) height growth in a provenance experiment some preliminary results
- Irbe I., Grīnfelds U., Šāble I., Verovkins A., Vīķele L., Horste B., Treimanis A., Škute M., Jansons Ā. Differences in wood traits between Norway spruce
- Kānberga-Siliņa K. Genetic determination of wood traits in Scots pine – analysis of candidate genes influencing wood quality
- Zeps M. Genetic diversity with aspen stands: phenology observations and molecular analysis
- Danusavicius D., Jansons Ā. Genetic variation in annual height growth of Latvian Norway spruce OP families
- Voronova A., Ruņģis D., Jansons Ā. Genome plasticity of Scots pine (*Pinus sylvestris* L.) under different stress conditions
- Irbe I. Spruce. Natural durability
- Maaten T. Within stand relatedness and genetic diversity
- Zeps M., Grīnfelds U. Wood trait differences of Hybrid aspen clones

Konferences ietvaros diskutēti pētījumu metodiskie aspekti un nepieciešamie papildus paraugu ievākšanas vai apstrādes darbi, lai visus iegūtos rezultātus būtu iespējams sagatavot zinātnisku publikāciju formātā. Tāpat analizēti rezultātu praktiskās izmantošanas aspekti.

Genetic variation of adaptive traits

Salaspils, LSFRI Silava, 30.11.2011.

9:00 Predicted climatic changes and possibilities for adaptation
9:40 Within-stand relatedness and genetic diversity: review
10:00 Genetic diversity with aspen stands: phenology observations and molecular analysis
10:20 prospects of further analysis – discussion
11:00 break
11:20 annual shoot development dynamics of hybrid aspen: results of 2010, 2011
11:40 annual shoot development dynamics of Scots pine
12:00 annual shoot development dynamics of Norway spruce
12:20 climatic factors and annual height growth development of *Pinus contorta*: preliminary results
12:40 break
13:40 productivity of *Pinus contorta* in Lithuania
14:00 traits of *Pinus contorta* in Latvia and Estonia
14:20 influence of genetic factors and regeneration method on Scots pine productivity
14:40 influence on thinning and genetic factors on productivity and quality of young Silver birch stands
15:00 break
15:20 genetic differences in wood traits of hybrid aspen
15:40 below-ground biomass of hybrid aspen
16:00 genetic differences in stress resistance
16:20 genetic determination of wood traits
16:40 differences in wood traits between Norway spruce clones
17:00 discussion, closing remarks,
departure to Jaunkalsnava

Jaunkalsnava, 1.12.2011

9:00 genetic differences among Scots pine families in resistance against needle cast
9:20 genetic and environmental factors determining occurrence of lamas growth of Scots pine
9:40 differences among Scots pine families in resistance against root rot
10:00 differences among Norway spruce clones in resistance against wood decay fungi
10:20 prospects of further analysis – discussion
10:40 break
11:00 departure to trials
15:30 closing of the conference

ESF project “Importance of Genetic Factors in Formation of Forest Stands with High Adaptability and Qualitative Wood Properties” (No 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146)



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Annual shoot development dynamics of hybrid aspen: results of 2010, 2011.

Mārtiņš Zeps

martins.zeps@silava.lv



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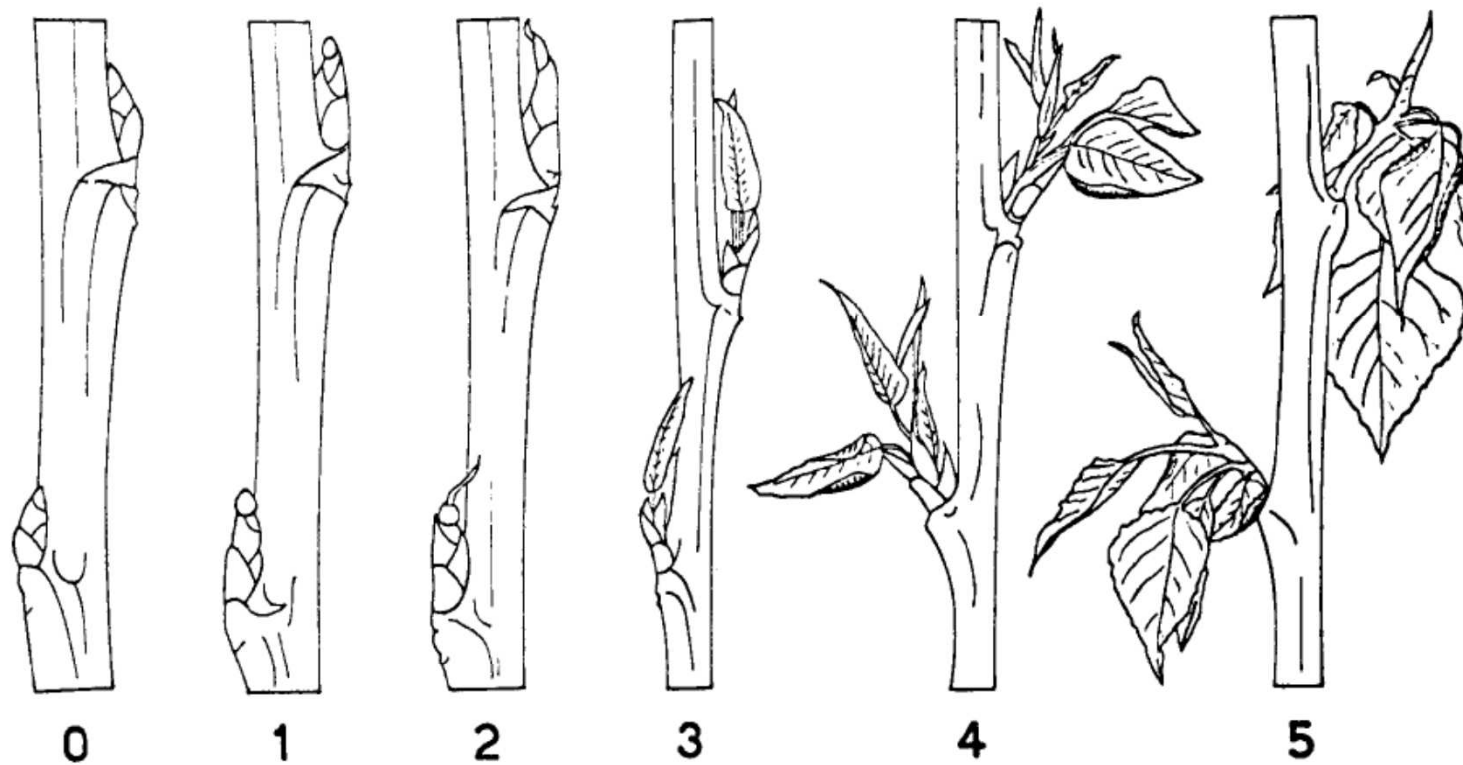
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Materials and method



Progeny trial No. 620 with 15 hybrid aspen clones, represented by 25 ramets with initial spacing 3 x 3 m, has been established in former agricultural land in central part of Latvia. Inventory of phenology and measurements have been carried out in 4th and 5th growing season, starting from middle of April, with an interval of 1 week on average.

Materials and method



Leaf prosperity phases



Differences between leaf prosperity was 12 day in phase 2 in 2010

But in 2011. only 2 days.

Differences between leaf prosperity was 4 days in phase 5 in 2010

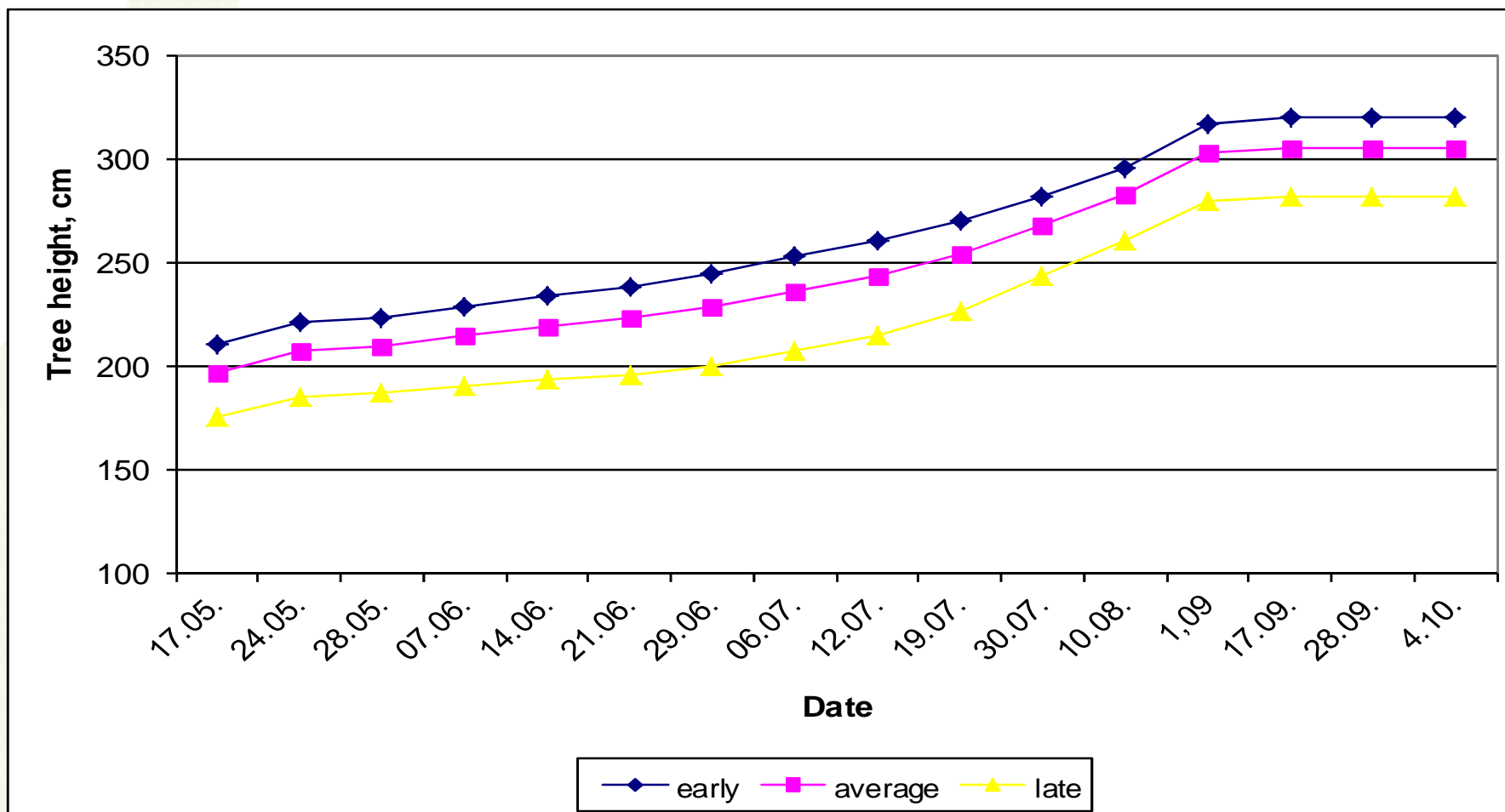
But in 2011. 7 days.



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Development of tree height increment with different bud burst phenology pattern 2010

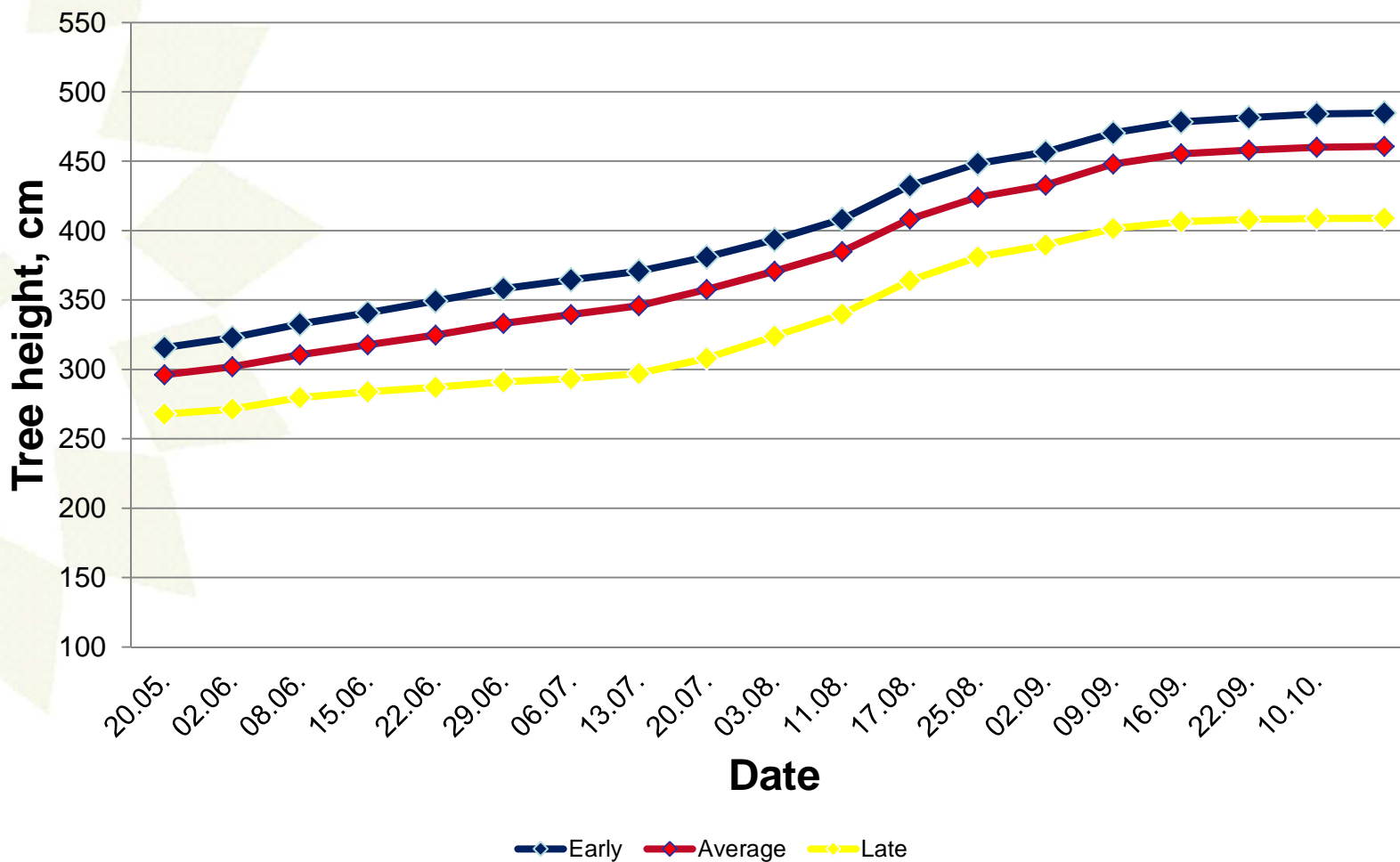




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Development of tree height increment with different bud burst phenology pattern 2011

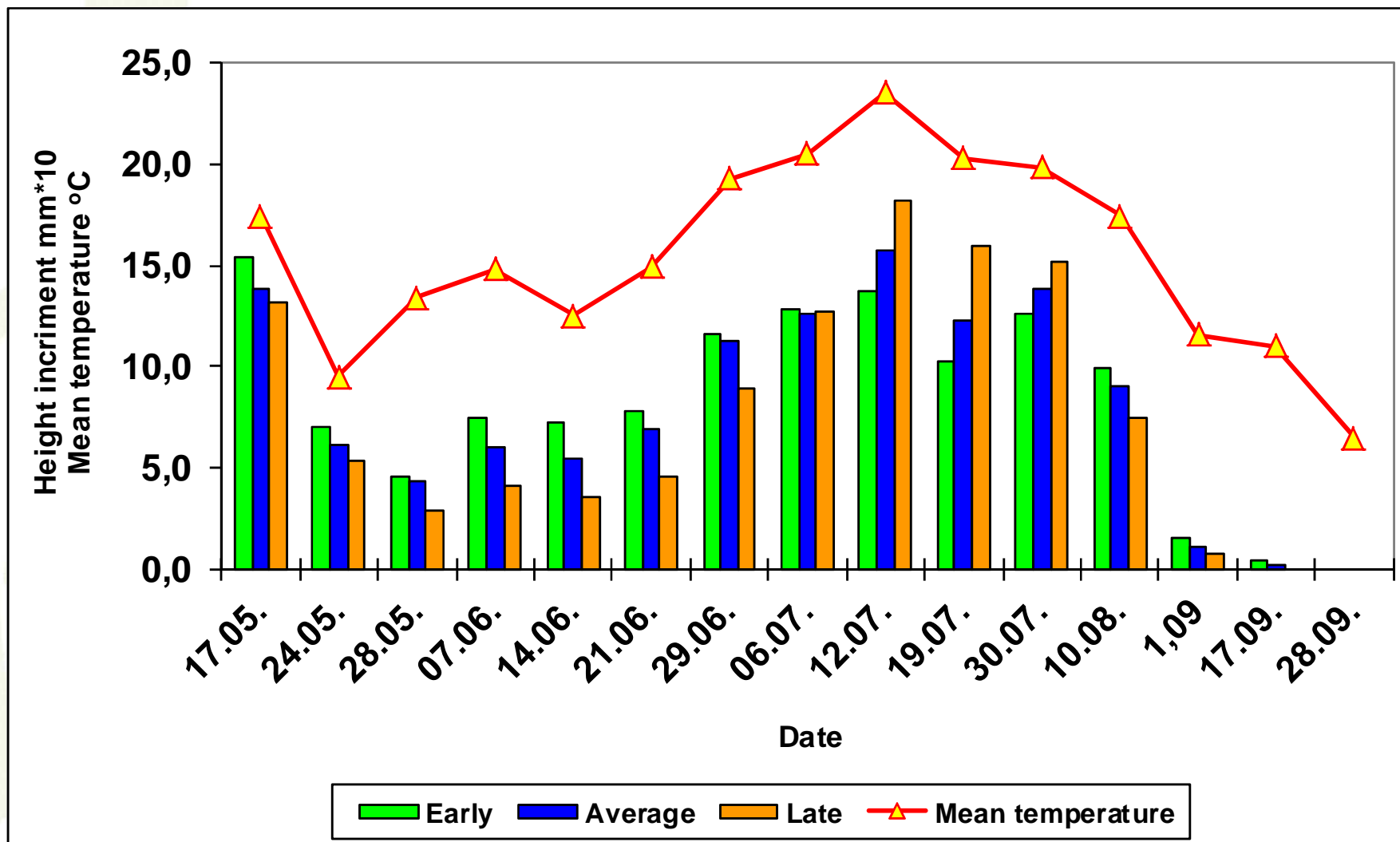




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Intensity of height growth per day, mm *10, of clones with different bud burst and mean temperature °C 2010.

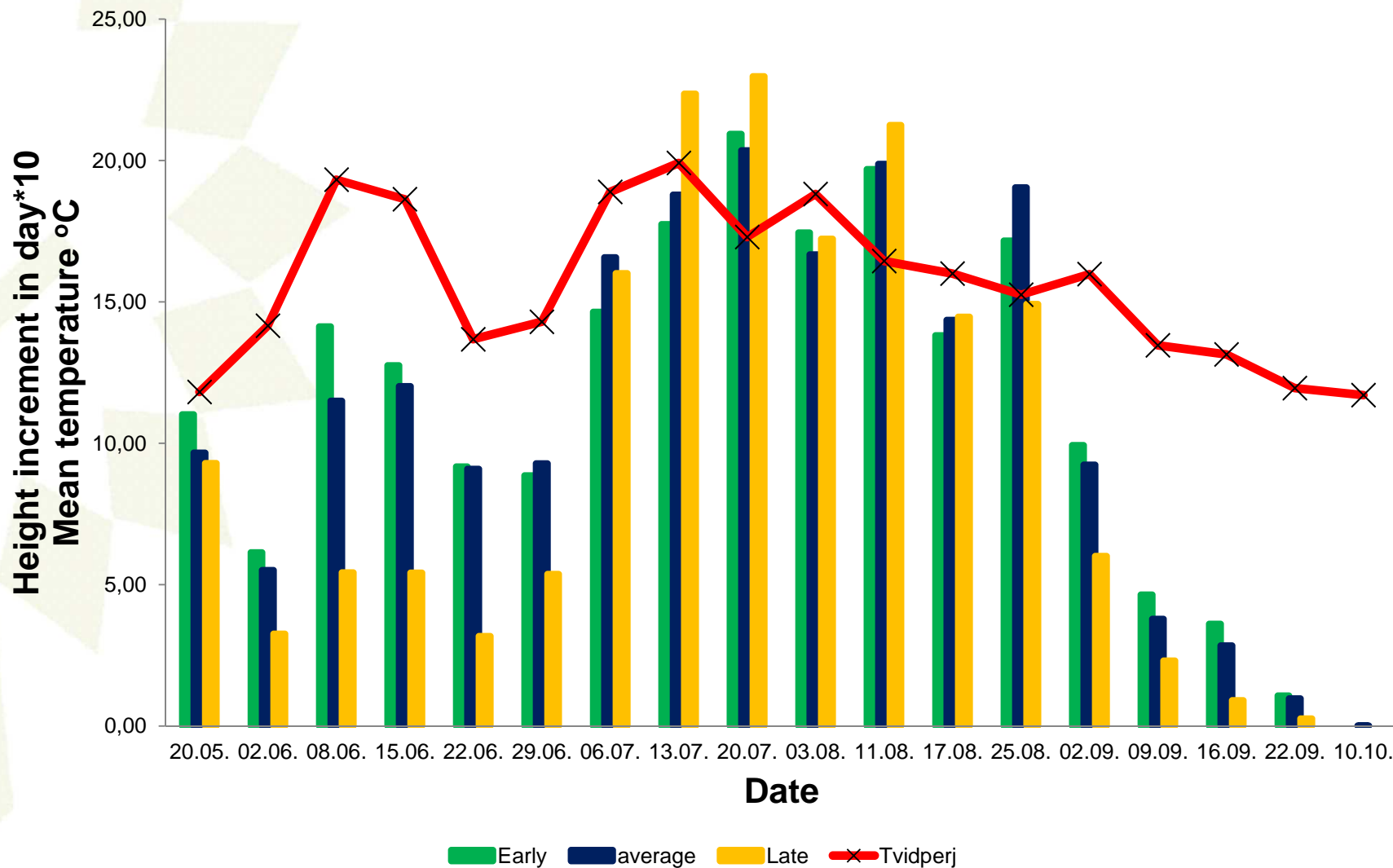




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Intensity of height growth per day, mm *10, of clones with different bud burst and mean temperature °C 2011

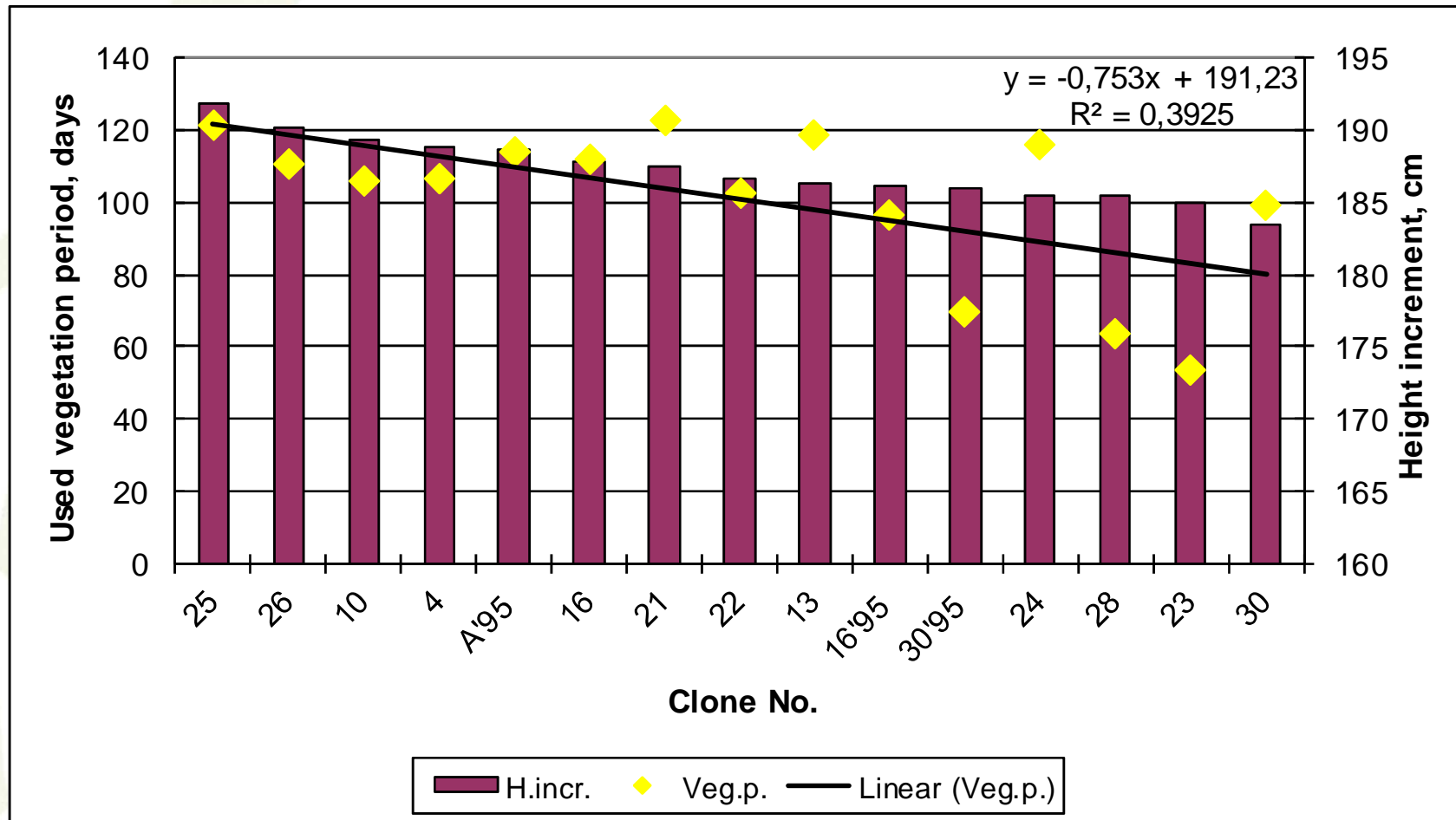




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Total length of height increment and length of used vegetation period for particular hybrid aspen clones 2010

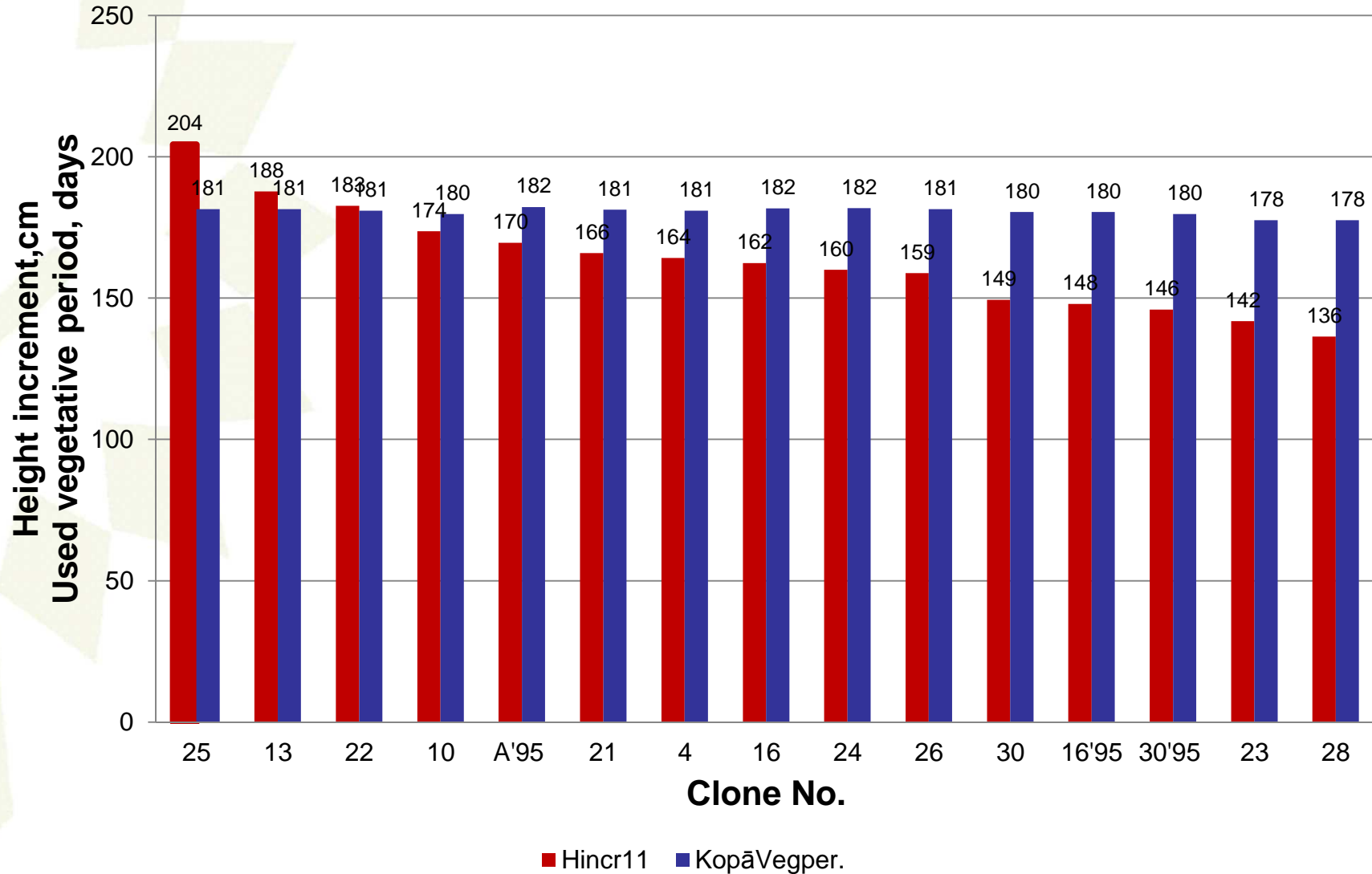




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Total length of height increment and length of used vegetation period for particular hybrid aspen clones 2011





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Conclusions



- Hybrid aspen clones have 2 height increment culminations – end of May and middle of July;
- Height increment have strong correlation this average temperature of day;
- Clone who have longer used vegetation period have bigger height increment;
- Growing intensity have bigger influence to total height increment of middle of July in 2010, but in 2011 midle of June.

Thank You for attention!



Nr. 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146



Annual shoot development dynamics of Scots pine

(genetic differences)

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Repeated measurements of height carried out in 3 trials:

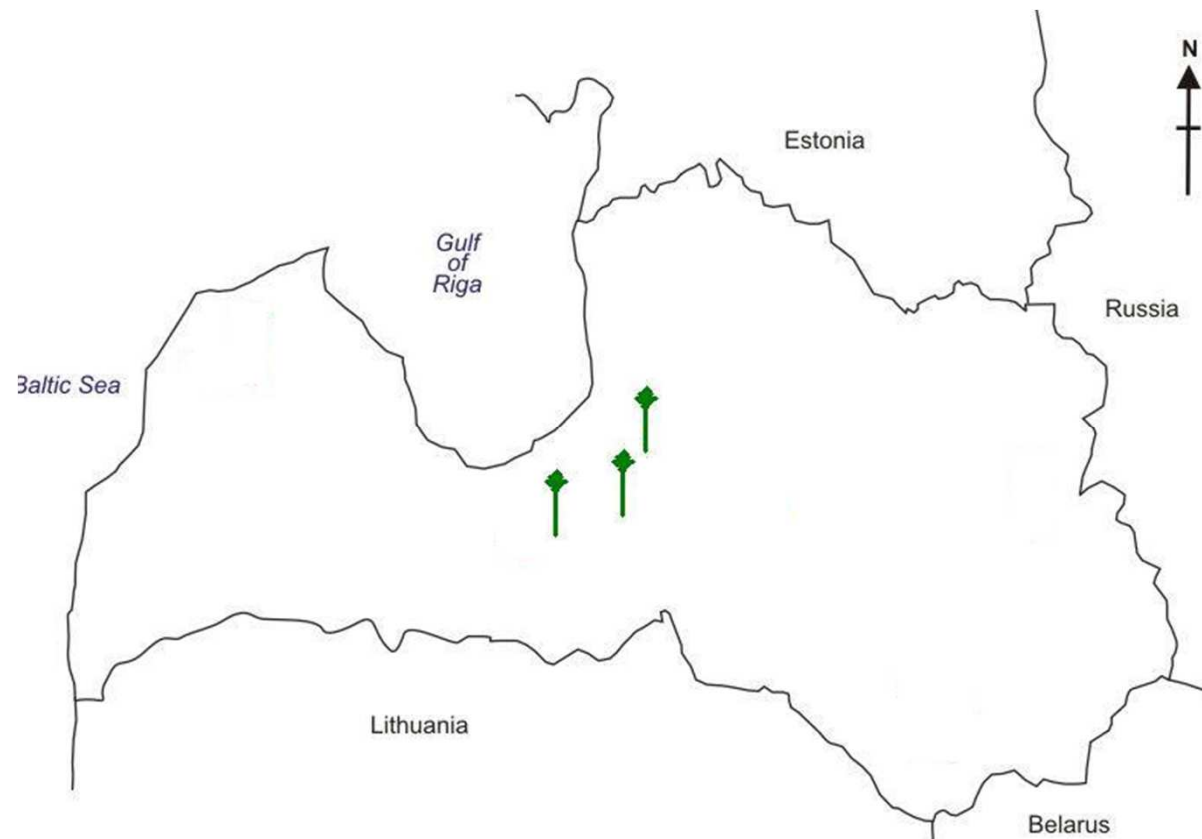
- Grafted seed orchard, age 10 years
- In total 72 clones, represented by 4 ramets (copies) each
- Mean height 4.1 m, diameter 7.2 cm
- Height differences for 95% of ramets does not exceed 0.4 m from mean and have no significant influence to annual height increment ($r^2=0.03$) or height growth intensity ($r^2=0.02$)
- Measurements carried out in year 2008



- Open pollinated progeny trials (experiments No. 352 and 441) at the age of 4 and 6 years (in year 2008 and 2010)
- Progenies of 61 tree and 4 control lots
- Mean height at the beginning of 6th vegetation period in exp. No. 352 was 110.5 cm and in exp. No. 441 – 64.0 cm.



- All experiments located in poor sandy soil, *Vacciniosa* forest type in relative similar climatic conditions, surrounded by Scots pine stands



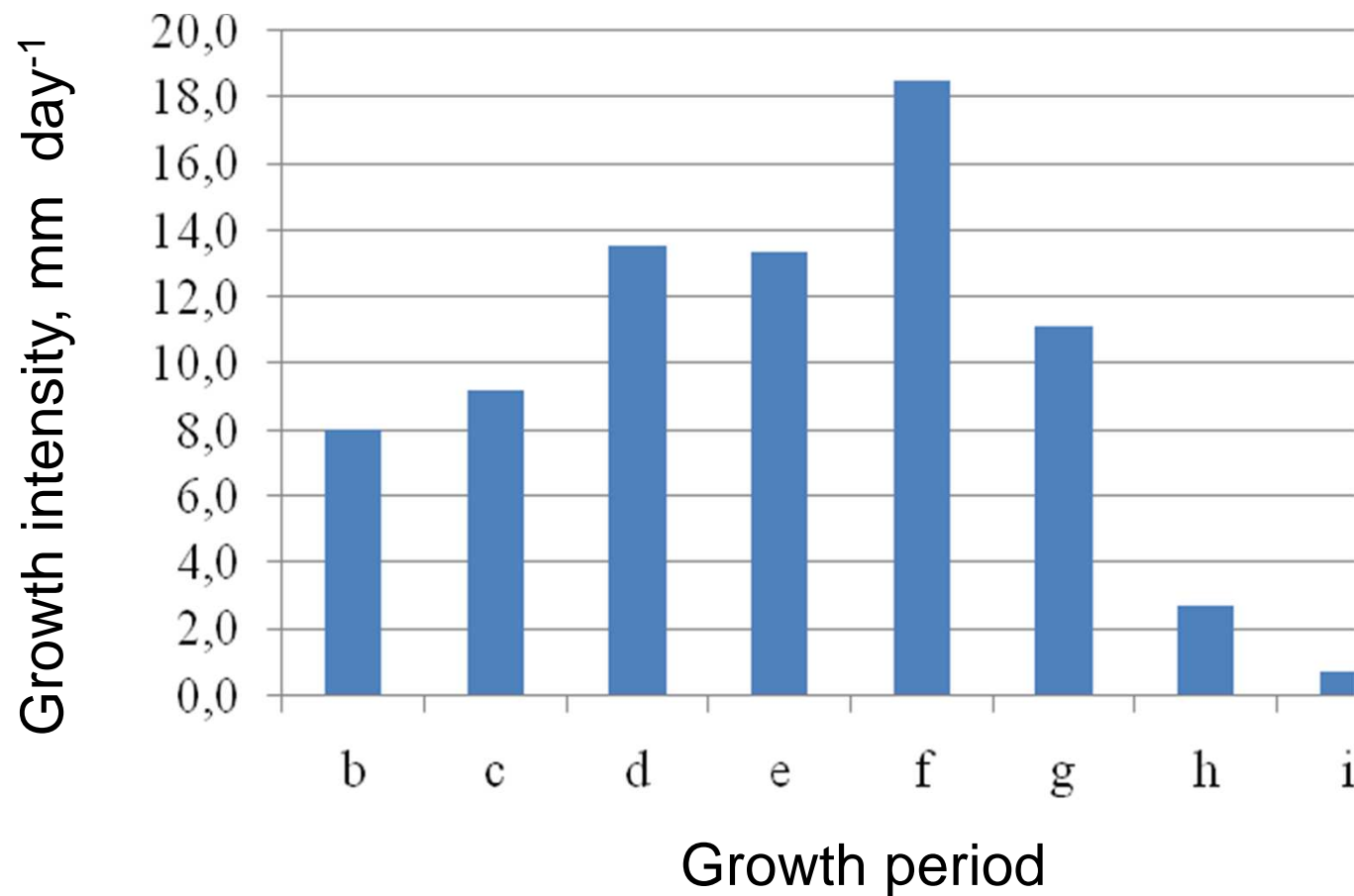
Results

Seed orchard



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- Average height increment reached 70 cm

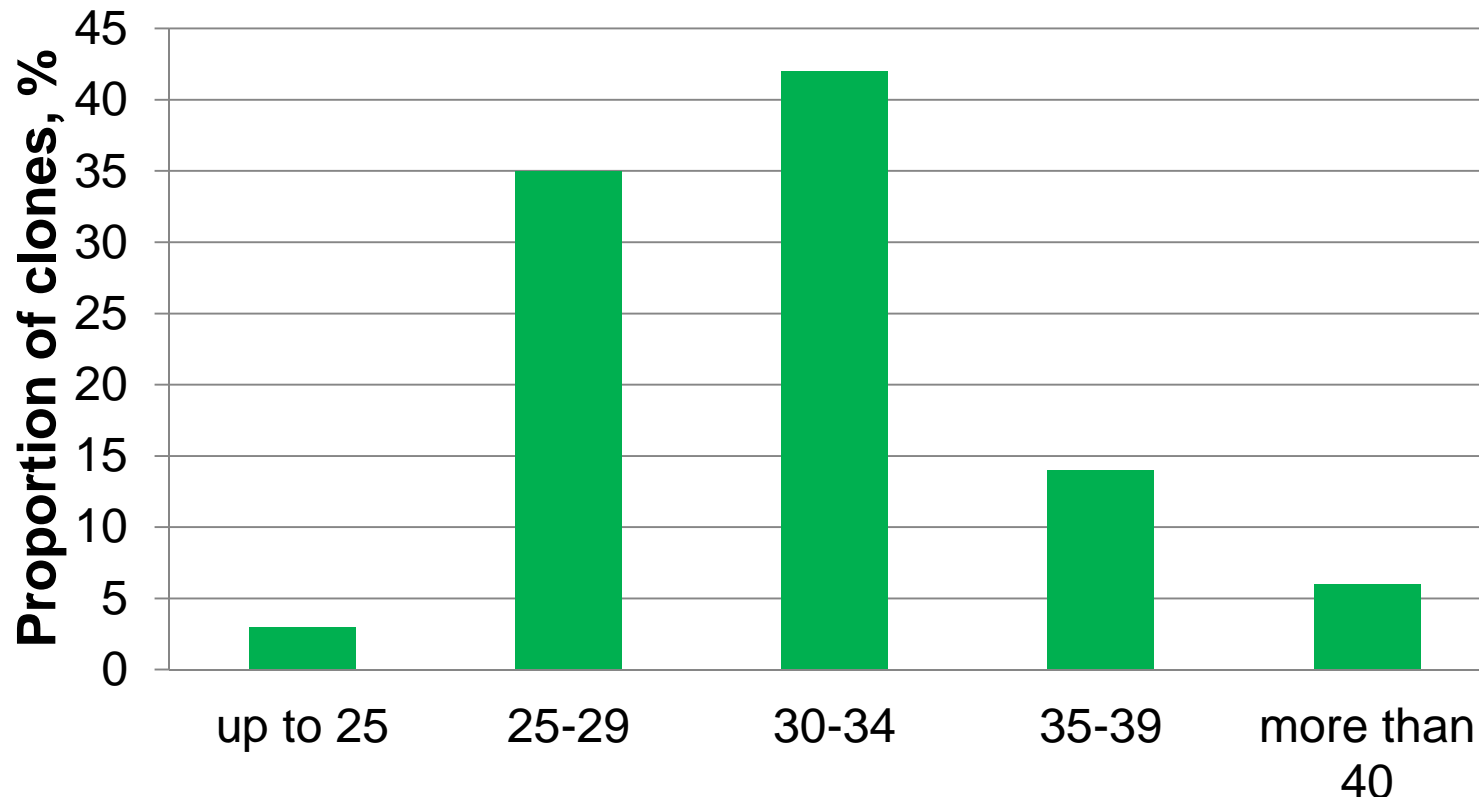


Growth period: a- 27.04.-04.05.; b-05.05.-10.05.; c-11.05.-16.05.; d-17.05.-22.05.; e- 23.05.-30.05.;f-31.05.-4.06.;g-5.06.-11.06.; h-12.06.-24.06.; i-25.06.-02.07.

Results Seed orchard



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Share of total height increment, formed in period with highest growth intensity, %

Share of total height increment, formed in period with highest growth intensity, has moderate heritability ($H^2=0,30$), similar to that of growth intensity on average ($H^2=0.29$)

Results Seed orchard



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- On average 32% of height increment is formed during the period of highest growth intensity (end of May-beginning of June).
- Correlation between share of height increment, formed in period with highest growth intensity and total length of height increment is negative, non-significant and weak ($r=-0.22$).
- It is possible to select clones, forming more than 35% of total length of height increment during relative short period (15-20% of total height growth period) and still having average or high total length of height increment.

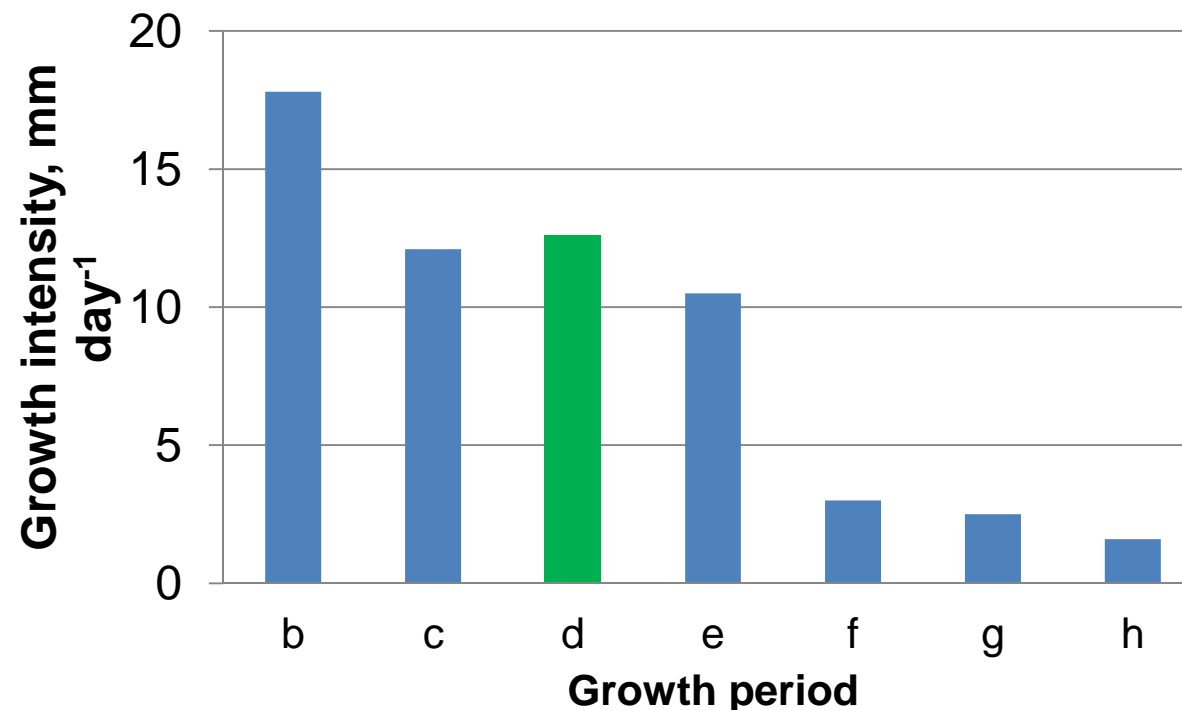
Results

O.p. progeny trials



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- Tree height have a relative large influence to height increment: correlation with total length of increment $r=0.56$ at the age of 4 years and $r=0.48$ at the age of 6 years.
- Average height increment reached 48 cm in exp. No. 352 and 23 cm in exp. No. 441.



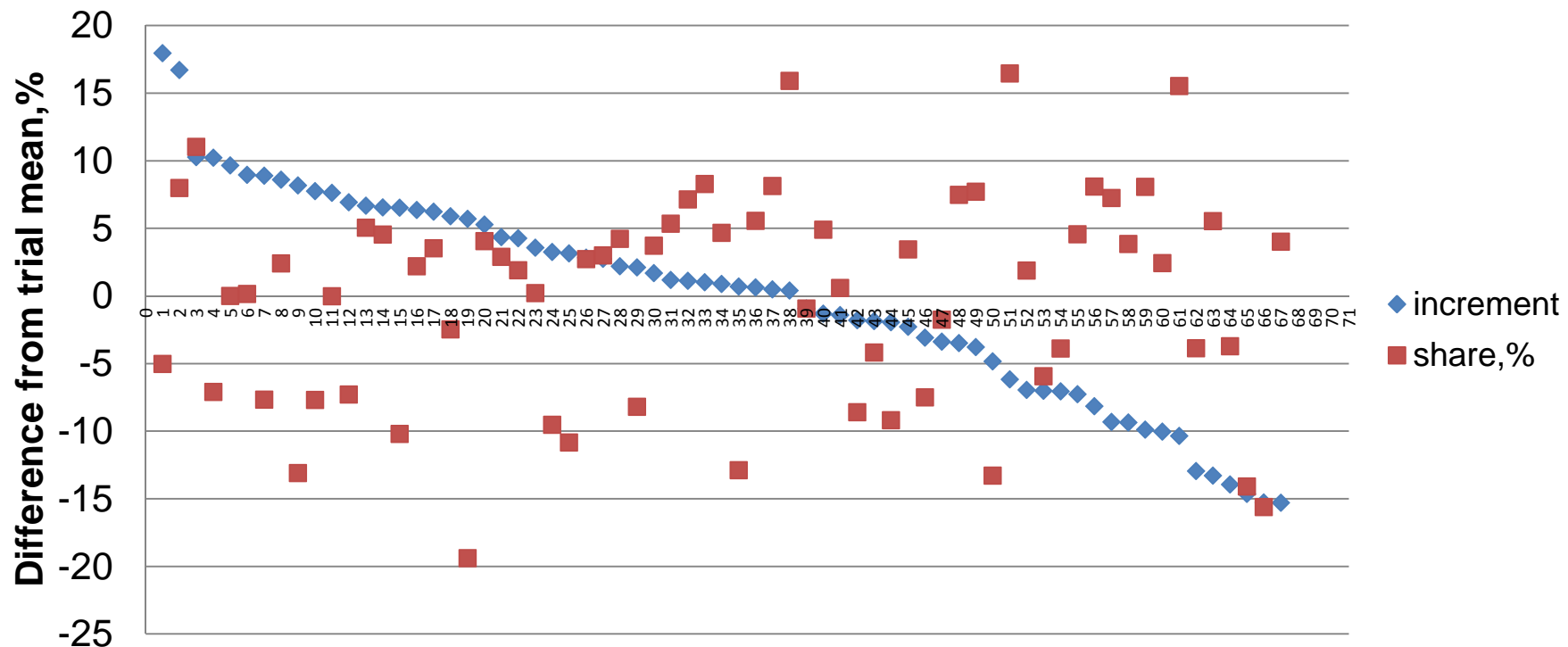
Results

O.p. progeny trials



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- In both trials and ages total length of height increment was weakly related to share of total height increment, formed in period with highest growth intensity (on average $r=-0.22$ at individual tree and $r=-0.07$ at family mean level).



share,% - share of total height increment, formed in period with highest growth intensity

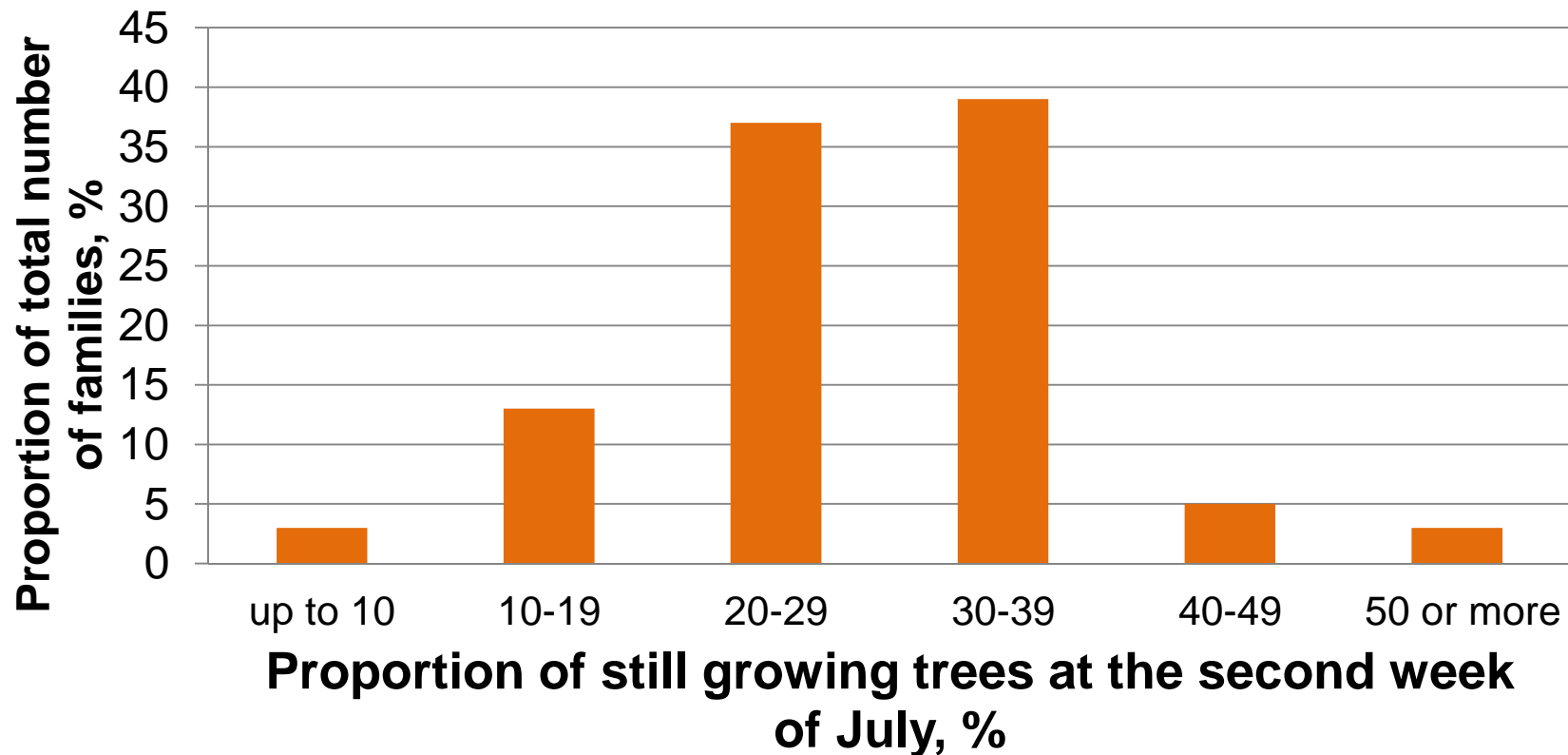
Results

O.p. progeny trials



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- Length of used growing season at family mean level was weakly related with total length of height increment ($r=-0.05$) and share of total height increment, formed in period with highest growth intensity ($r=-0.13$)



Conclusion



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Main conclusion – it is possible to select genotypes, forming relative large proportion from total increment in short period of time (1-2 weeks, corresponding to 15-20% of height growth period), but selections especially for this trait needs to be carried out; ordinary selection based on tree height would not yield the result





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Parts of the studies have been funded by:

- Forest Development Fund (2009)
- ESF project “Importance of Genetic Factors in Formation of Forest Stands with High Adaptability and Qualitative Wood Properties”
(No. 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146)

Thank You!

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Climate impacts on lodgepole pine (*Pinus contorta* var. *latifolia*) height growth in a provenance experiment- some preliminary results

Zane Libiete-Zalite, LSFRI “Silava”

Some theoretical background (1)



- Lodgepole pine (*Pinus contorta*, including all subspecies) is widely spread throughout western North America, growing between the latitudes 30° and 64°N.
- Lodgepole pine has a wide ecological amplitude, its varieties being adapted to maritime, continental and subalpine conditions and capable of growing on any type of site.
- There are three main varieties: a coastal form (var. *contorta*), a southern inland form (var. *murrayana*) and a northern inland form (var. *latifolia*).
- The *latifolia* variety has proved useful in northern European conditions, combining fast and straight growth with sufficient climatic hardiness; around 1970 large-scale introduction of these species was started in Sweden.

Some theoretical background (2)



According to results of Scandinavian experiments,

- The wood quality of lodgepole pine is comparable to that of Scots pine (*Pinus sylvestris* L.) grown under similar conditions;
- Lodgepole pine has slightly lower wood density, lower bark proportion in stem volume, higher proportion of heartwood and better stem form than Scots pine;
- Lodgepole pine is estimated to produce up to 36% more yield than Scots pine, irrespective of the site index; the optimum rotation is 10-15 years shorter;
- Survival during the initial stand development is higher for lodgepole pine than for Scots pine;
- Lodgepole pine plantations are less stable and lose more biomass due to wind and snow damage than Scots pine.

Consequently...



- Lodgepole pine might be of interest for production of energy wood, pulpwood and perhaps also saw-timber in plantation forestry in Latvia

Understanding tree growth-climate relationships



- Before any recommendations for the use of lodgepole pine in Latvian forestry can be made, it is essential to understand how the tree growth is affected by weather and climate variables in Latvian conditions → ADAPTATION
- The most common way to analyze responsiveness of the growth to the climate variables is the analysis of radial growth patterns (tree-rings)
- Several studies suggest that height growth is more suitable for examining the effects of climatic variations, less frequent use of this variable is most likely associated with more laborious gathering of the data

Objective



- The objective of the study was to analyze the effects of climate variables (temperature, precipitation) on the height growth of lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) trees from three various provenances

Study material



- Information on annual height growth (cm) of 297 lodgepole pine trees of 3 proveniences – Pink Mountain, Fort Nelson, Summit Lake
- Location of plantation – Zvirgzde
- Year of plantation establishment – 1985
- Information about mean monthly temperature and monthly precipitation amounts from meteostation Bauska

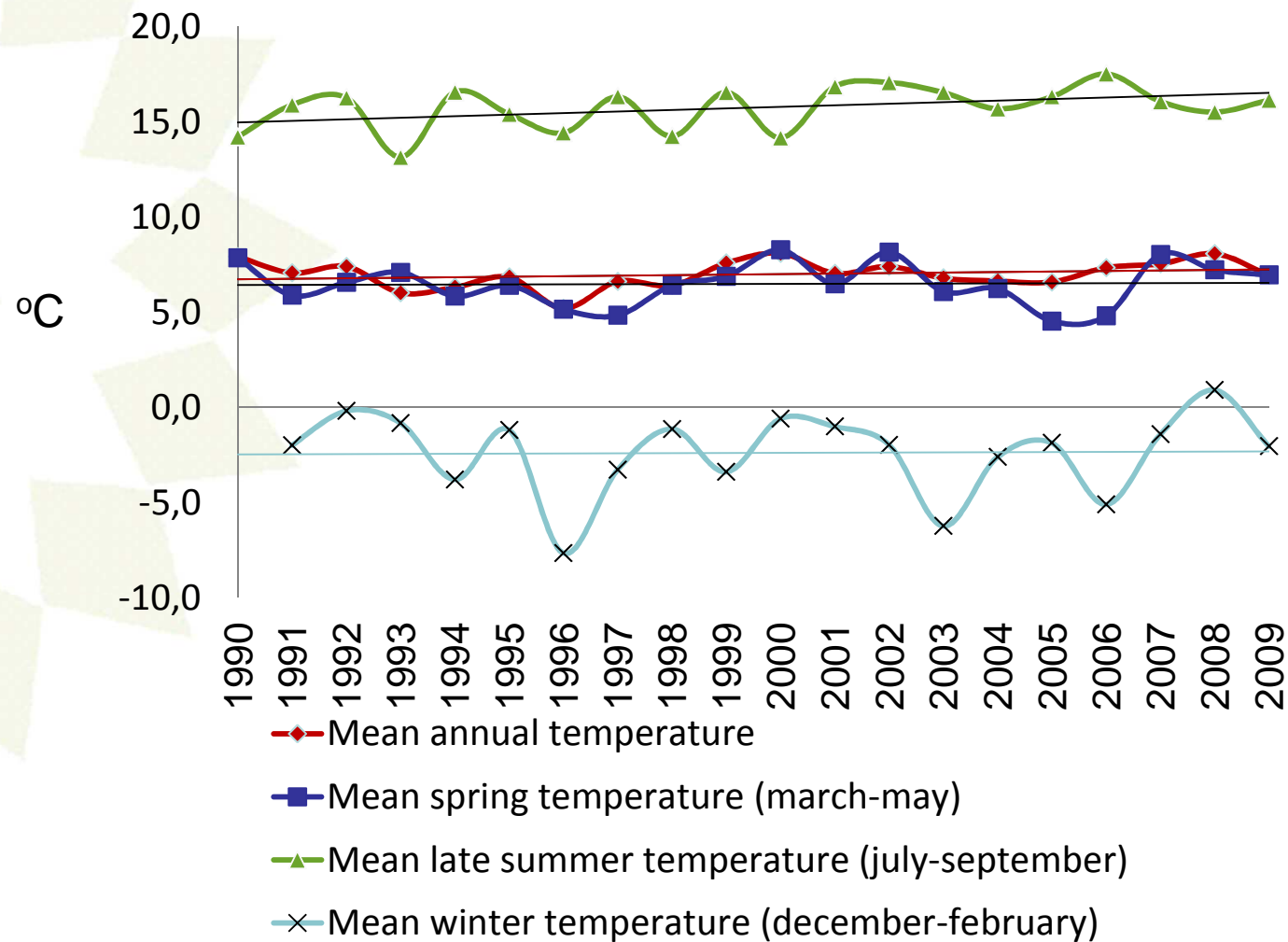


Whorl

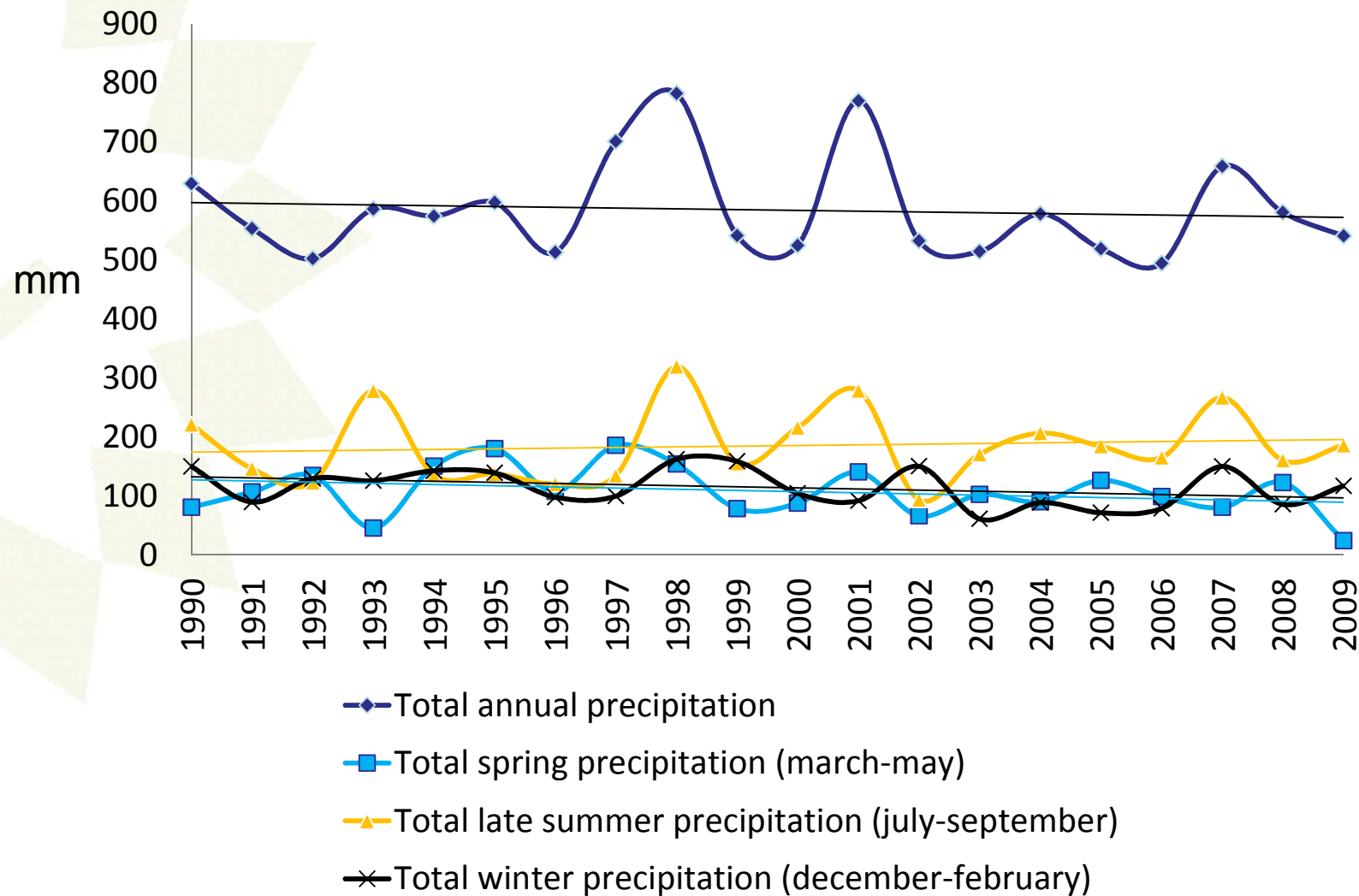
Secondary whorl

Provenance	Geographical		
	latitude	longitude	altitude a.s.l.
Pink Mountain	57°00'	122°15'-45'	850
Fort Nelson	58°38'	122°41'	495
Summit Lake	54°24'	122°37'	813

Temperature change



Precipitation change



Climate variables



Temperature variables

- Mean annual temperature
- Mean annual temperature of the previous year
- Mean late summer temperature of the previous year (July-September)
- Mean temperature from previous October to April of the current growing season
- Mean temperature of previous November-December

Precipitation variables

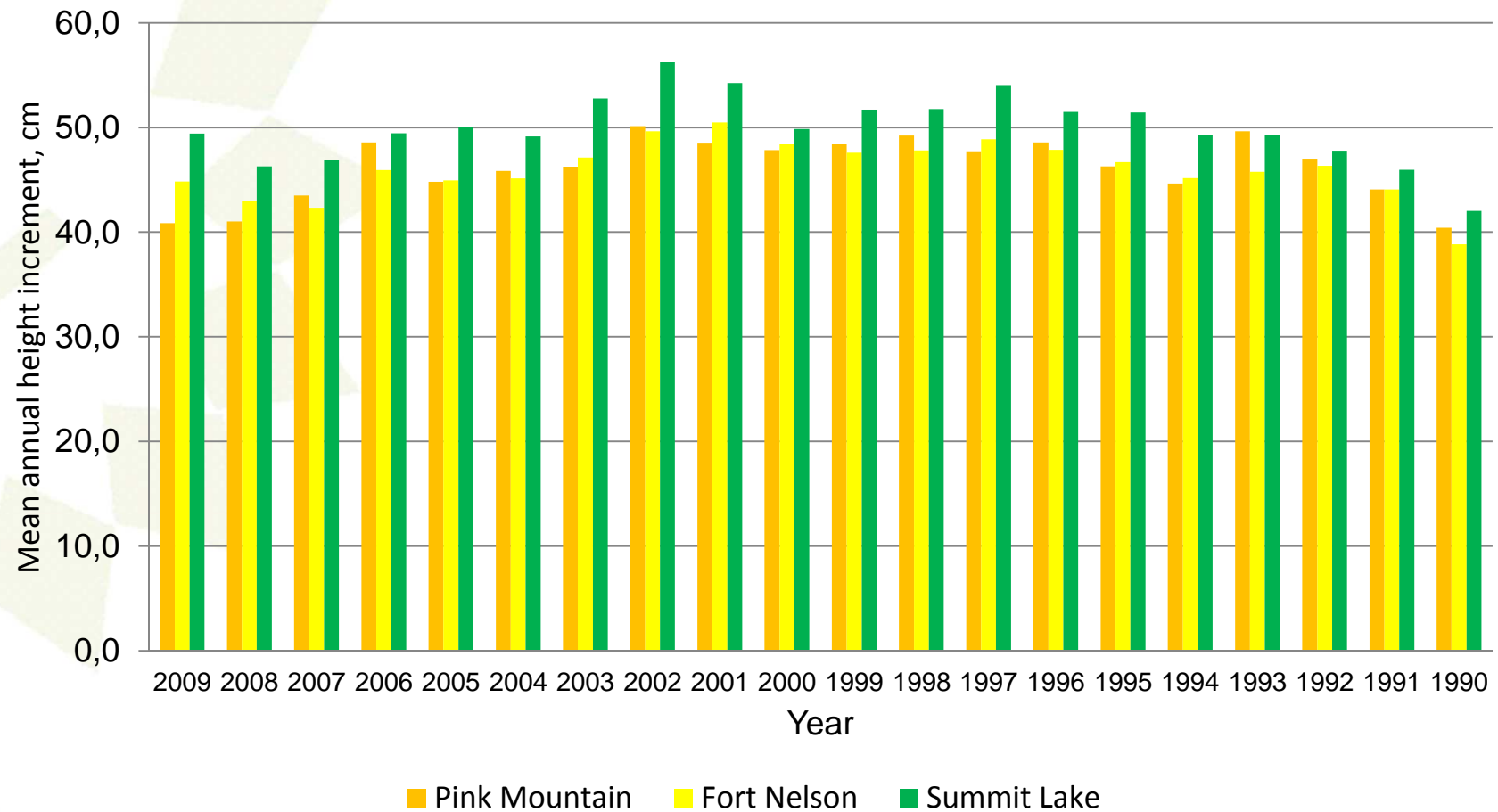
- Total annual precipitation
- Total annual precipitation of the previous year
- Total precipitation from previous October to April of the current growing season
- Total late summer precipitation of the previous year (July-September)
- Total precipitation from previous December to February
- Sum of total precipitation of previous and current growing season (May-August)

Analysis



- All tree height increment values prior to 1990 were excluded from the analysis to remove establishment-related growth effect
- Linear regression analysis was used to determine possible relationships between annual height growth and climate variables
- Analysis was performed separately for each provenance

Results – mean annual height increment



Results – correlation with climate variables – Pink Mountain



Climate variable	R	R ²	F	p
Mean t°	0.078	0.006	11.264	0.001
Mean t° of the previous year	0.087	0.008	13.912	0.000
Mean late summer (July, August, September) t° of the previous year	0.018	0.000	0.606	0.436
Mean t° from previous October to April	0.080	0.006	11.702	0.001
Mean t° of previous November and December	0.069	0.005	8.603	0.003
Total annual precipitation	0.015	0.000	0.420	0.517
Total annual precipitation of the previous year	0.011	0.000	0.235	0.628
Total precipitation from previous October to April	0.017	0.000	0.528	0.467
Total late summer (July, August, September) precipitation of the previous year	0.015	0.000	0.398	0.528
Total precipitation from previous December to February	0.016	0.000	0.459	0.498
Total precipitation of the previous and current growing seasons combined (May-August)	0.060	0.004	6.635	0.010

Results – correlation with climate variables – Fort Nelson



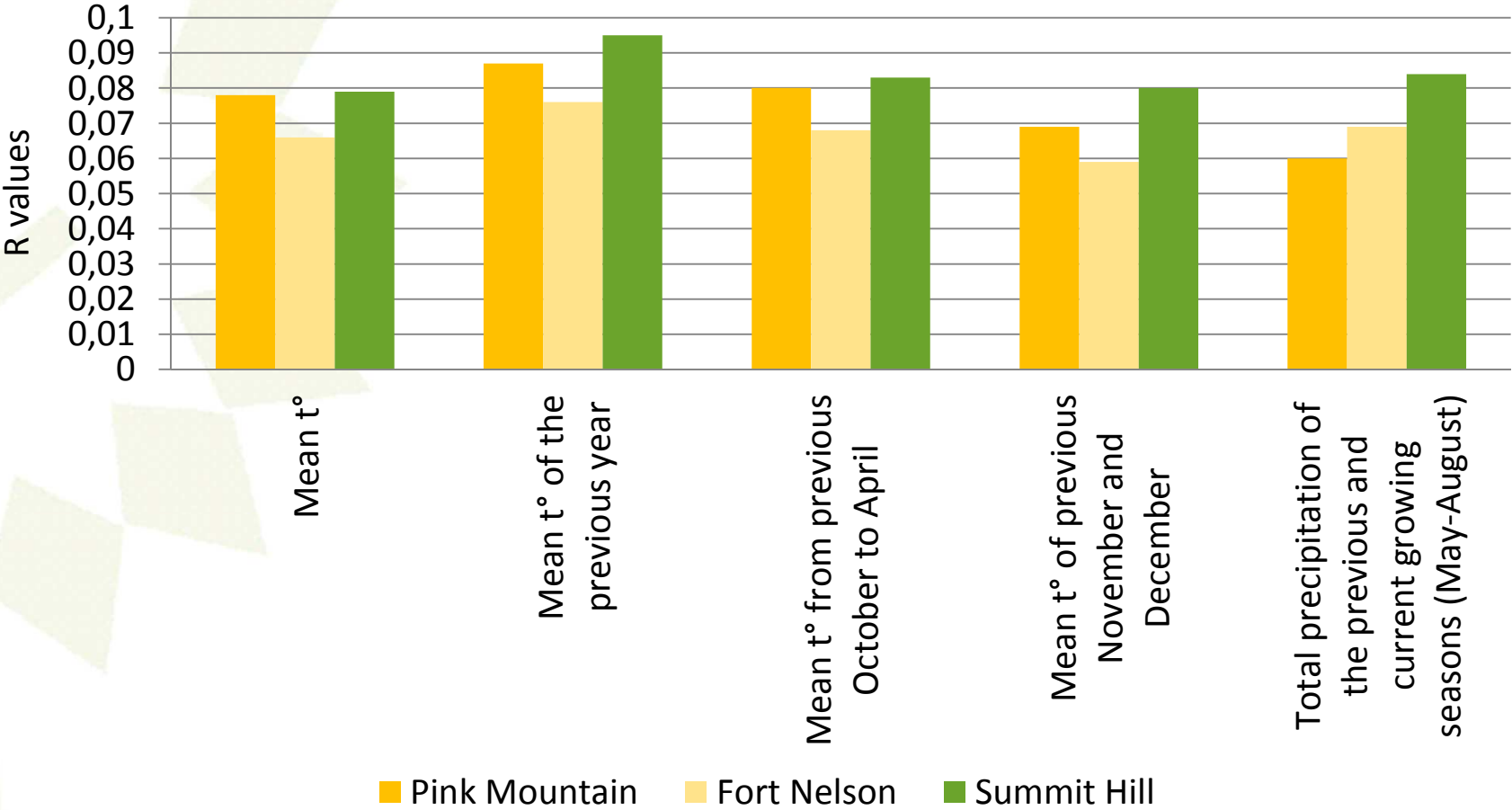
Climate variable	R	R ²	F	p
Mean t°	0.066	0.004	8.943	0.003
Mean t° of the previous year	0.076	0.006	11.653	0.001
Mean late summer (July, August, September) t° of the previous year	0.012	0.000	0.273	0.602
Mean t° from previous October to April	0.068	0.005	9.254	0.002
Mean t° of previous November and December	0.059	0.003	7.009	0.008
Total annual precipitation	0.025	0.001	1.251	0.263
Total annual precipitation of the previous year	0.011	0.000	0.232	0.630
Total precipitation from previous October to April	0.016	0.000	0.544	0.461
Total late summer (July, August, September) precipitation of the previous year	0.004	0.000	0.036	0.849
Total precipitation from previous December to February	0.024	0.001	1.175	0.278
Total precipitation of the previous and current growing seasons combined (May-August)	0.069	0.005	9.670	0.002

Results – correlation with climate variables – Summit Lake



Climate variable	R	R ²	F	p
Mean t°	0.079	0.006	13.121	0.000
Mean t° of the previous year	0.095	0.009	19.137	0.000
Mean late summer (July, August, September) t° of the previous year	0.004	0.000	0.033	0.855
Mean t° from previous October to April	0.083	0.007	14.649	0.000
Mean t° of previous November and December	0.080	0.006	13.675	0.000
Total annual precipitation	0.033	0.001	2.240	0.135
Total annual precipitation of the previous year	0.026	0.001	1.461	0.227
Total precipitation from previous October to April	0.017	0.000	0.605	0.437
Total late summer (July, August, September) precipitation of the previous year	0.009	0.000	0.158	0.691
Total precipitation from previous December to February	0.014	0.000	0.406	0.524
Total precipitation of the previous and current growing seasons combined (May-August)	0.084	0.007	14.793	0.000

Results – comparison of correlation coefficients



Things to do next



- Get meteodata from a closer location, if possible;
 - Extend the analysis, including combined climate variables of temperature and precipitation
 - Compare the climate responsiveness of the lodgepole pine height growth to that of the Scots pine in Latvia
-



Thank You for Your attention!

European Social Fond project (No.
2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146)



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Differences in wood trials between Norway spruce

Ilze Irbe, Uldis Grīnfelds,
Inese Šāble, Anrijs Verovkins,
Laura Vīķele, Baiba Horste,
Arnis Treimanis, Mārīte Škute,
Āris Jansons e.t.c.



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Background



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Sample trees cut at age 21 in one site – Madona region

Wood used for analysis of the trunk from 0.5 to 1.3 meters in height from the ground.

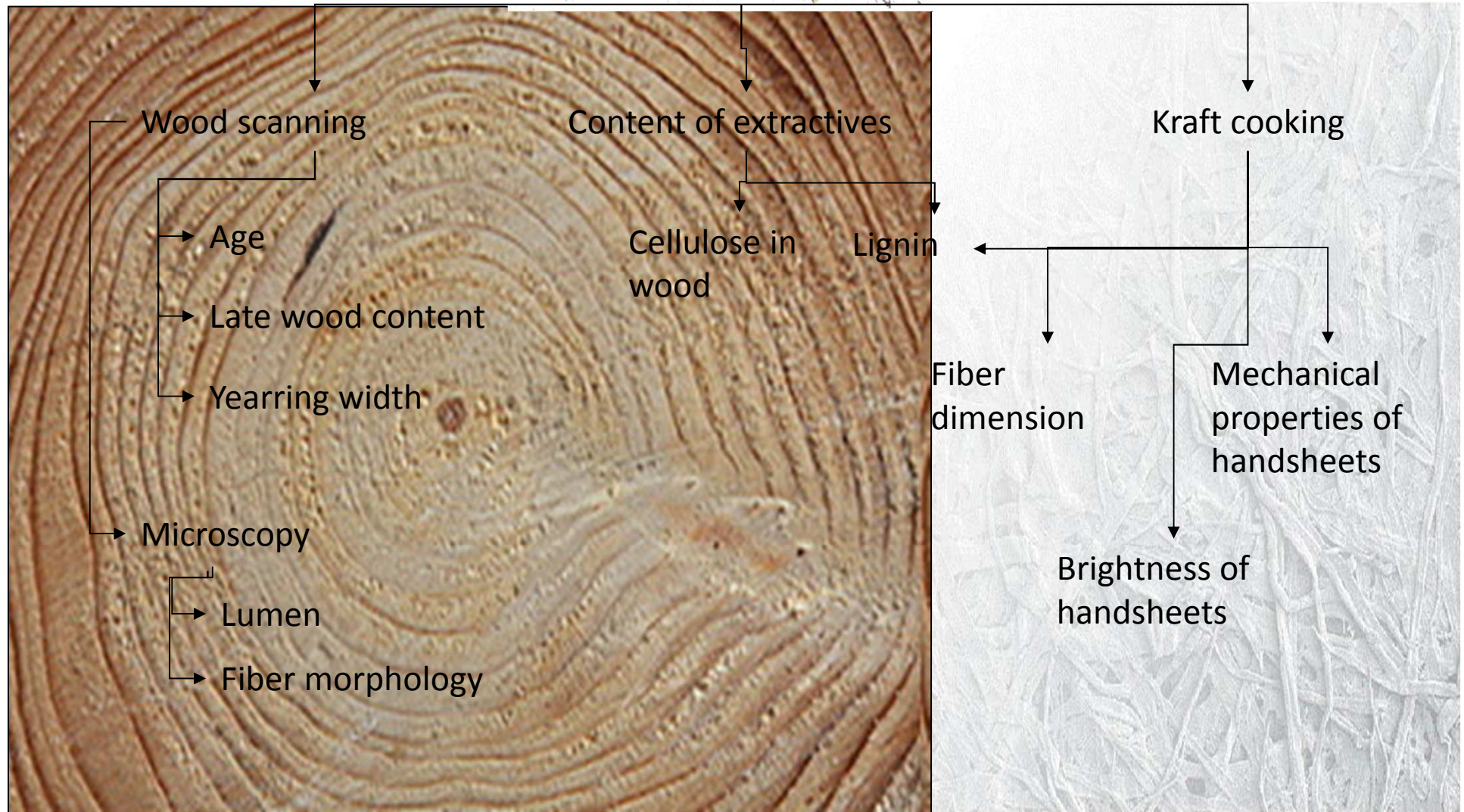
Clone	Count of trees
26	3
31	4
A10	3
A15	3
A7	2
B10	3
B15	3
B6	4
KR13	3
V7	3
V9	3



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Materials and method





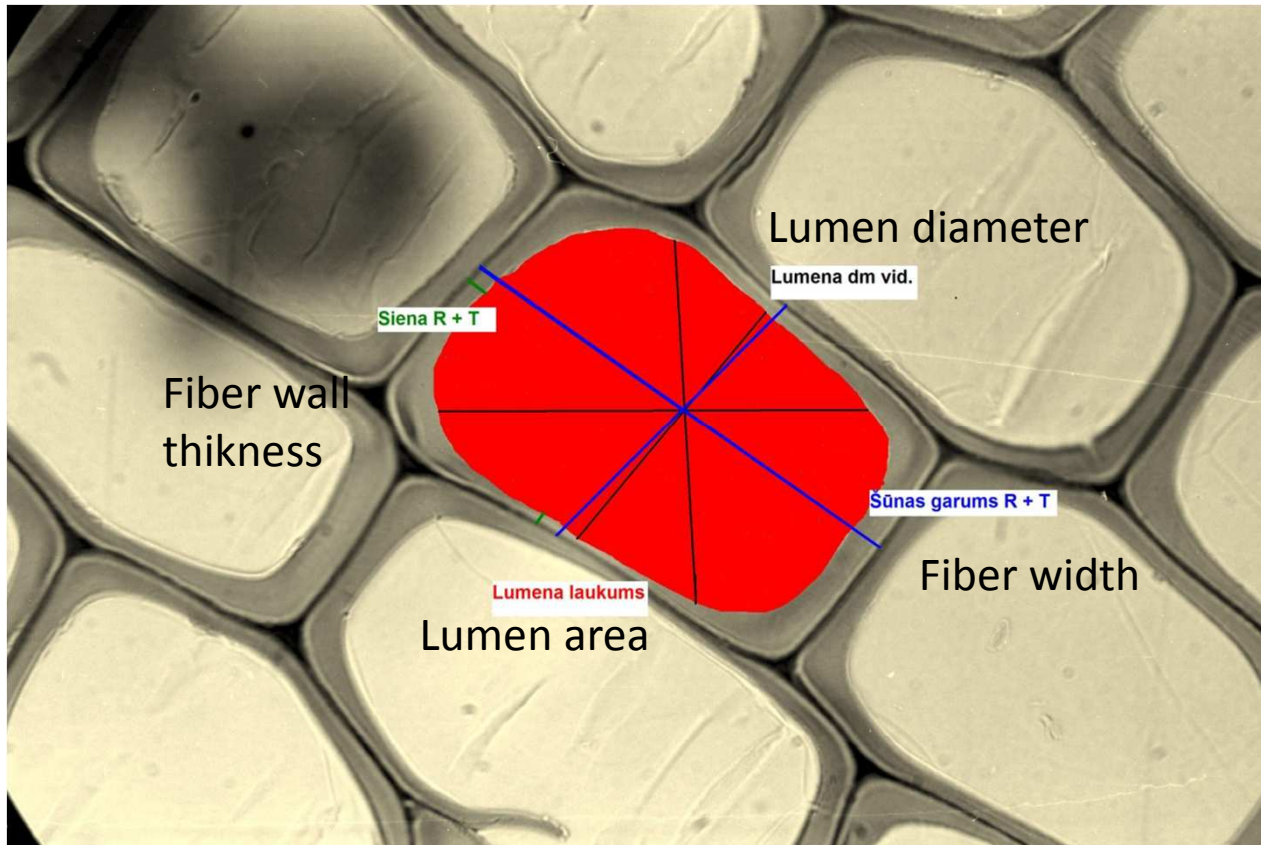
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Microscopy



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Results



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ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Stem diameter	Between Groups	485,497	10	48,550	12,551	,000
	Within Groups	208,879	54	3,868		
	Total	694,377	64			
Stem height	Between Groups	124,090	10	12,409	10,145	,000
	Within Groups	66,050	54	1,223		
	Total	190,139	64			
Cellulose content	Between Groups	18,383	10	1,838	1,340	,233
	Within Groups	76,814	56	1,372		
	Total	95,196	66			
Extractives in wood	Between Groups	6,509	10	,651	9,086	,000
	Within Groups	4,011	56	,072		
	Total	10,520	66			
Late wood content	Between Groups	519,623	10	51,962	12,628	,000
	Within Groups	213,965	52	4,115		
	Total	733,588	62			
Density	Between Groups	86006,380	10	8600,638	19,605	,000
	Within Groups	23689,180	54	438,689		
	Total	109695,560	64			
Lignin in wood	Between Groups	22,467	10	2,247	5,794	,000
	Within Groups	21,716	56	,388		
	Total	44,184	66			



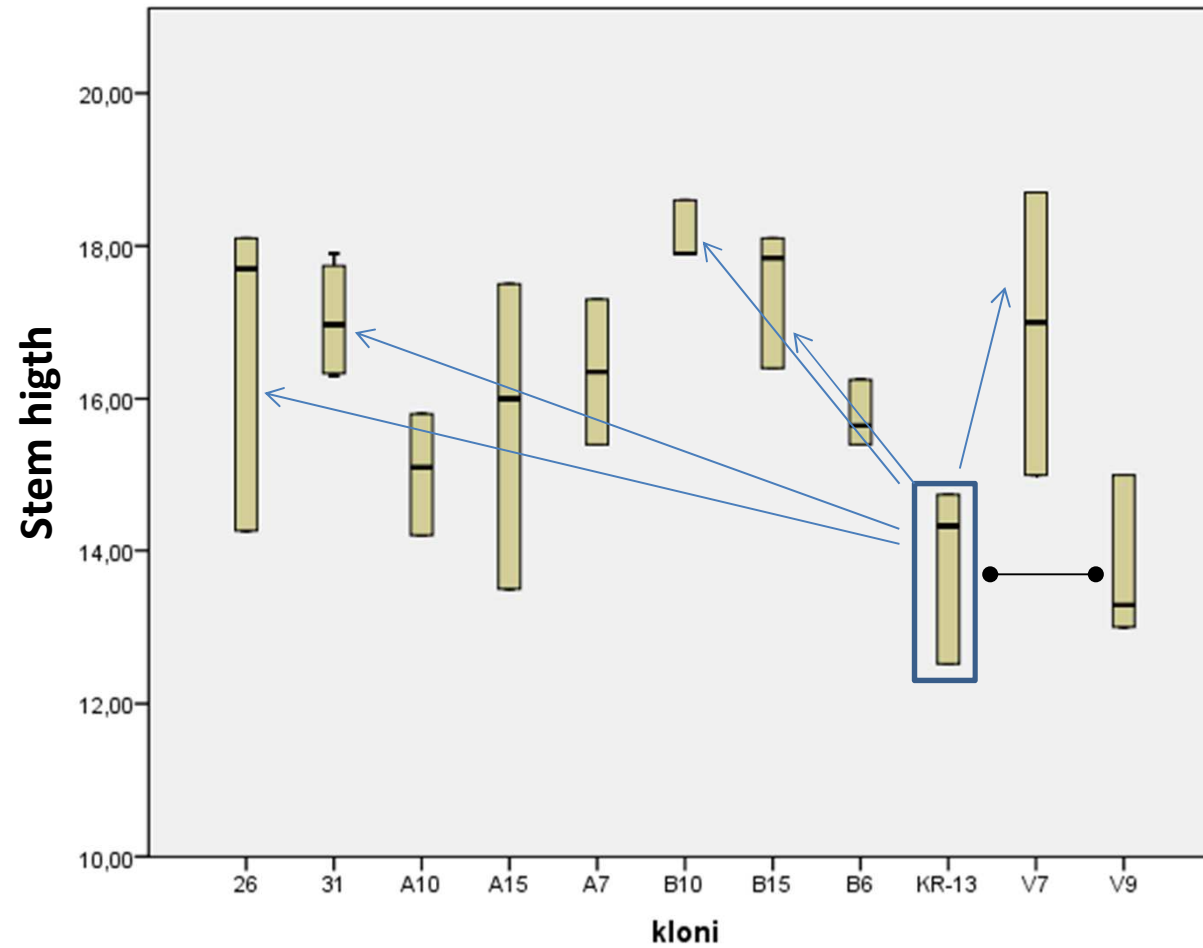
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INVESTMENT IN YOUR FUTURE

Results



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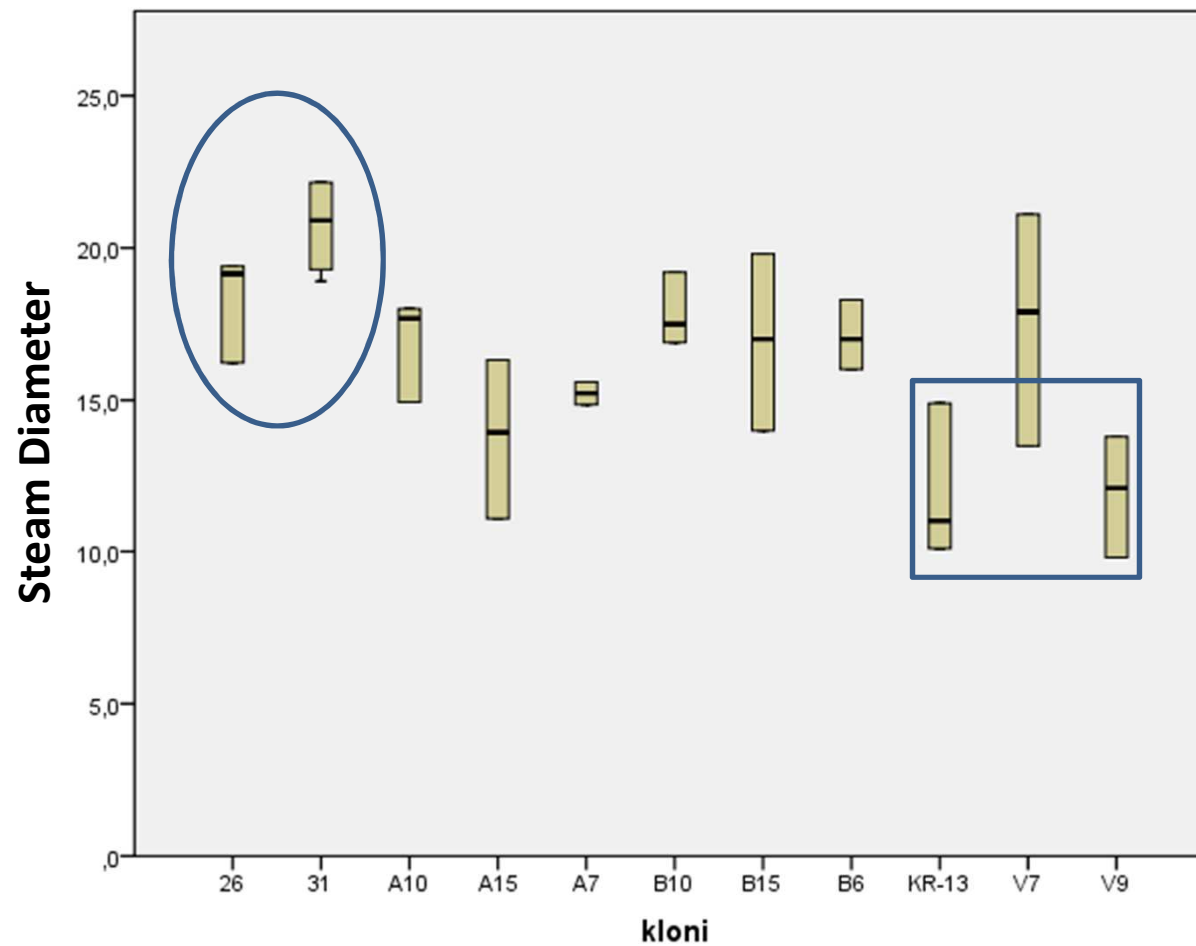
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INVESTMENT IN YOUR FUTURE

Results



SILAVA





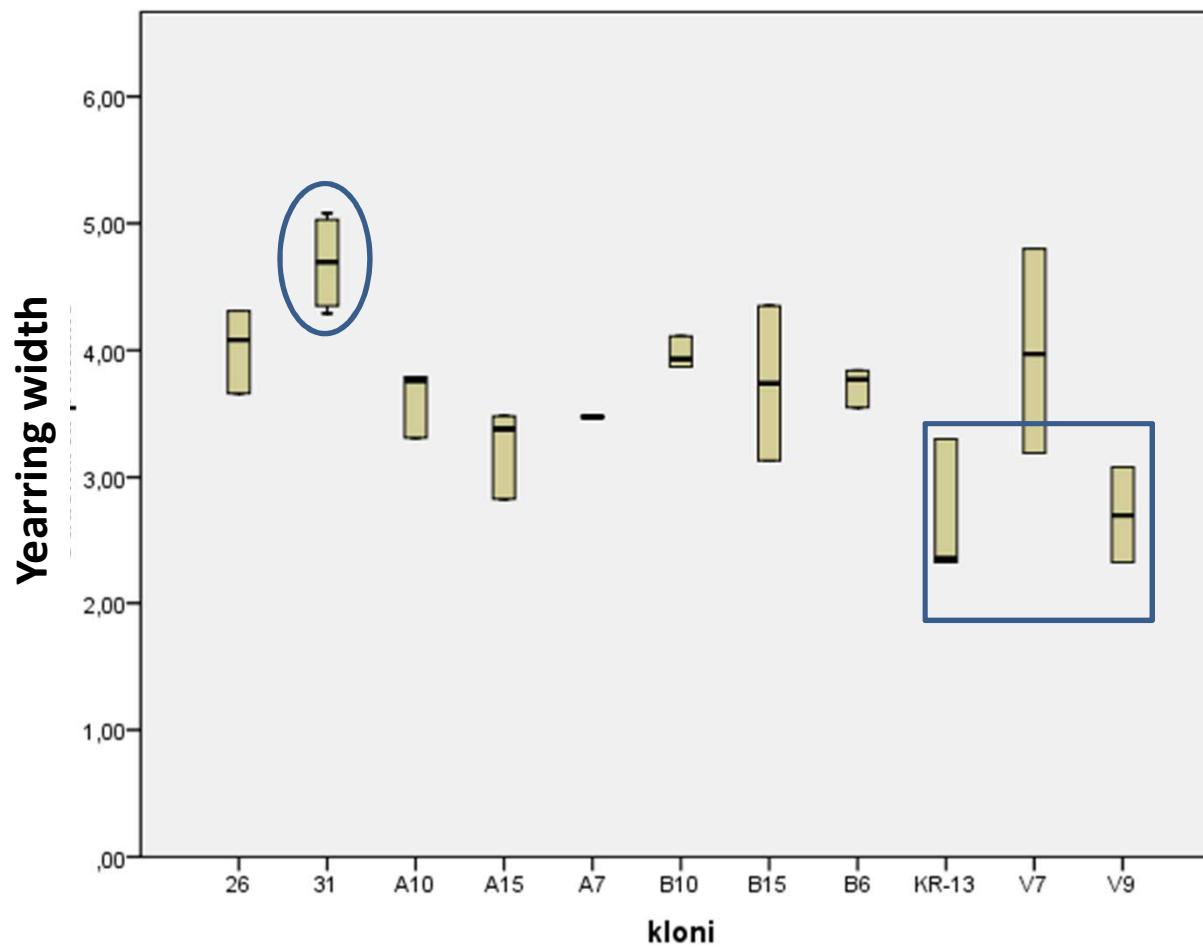
Funded by the European Union

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Results



SILAVA





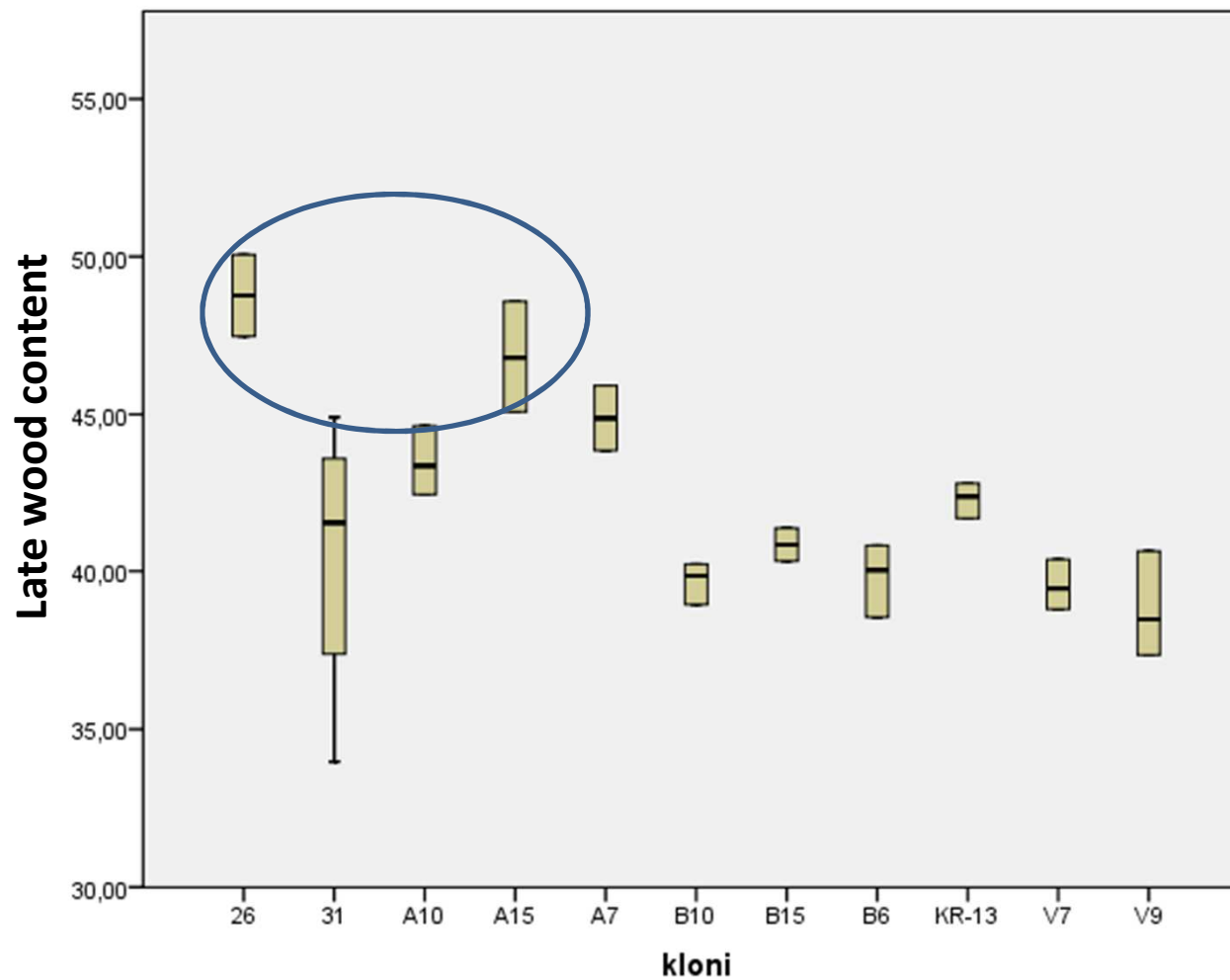
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Results



SILAVA





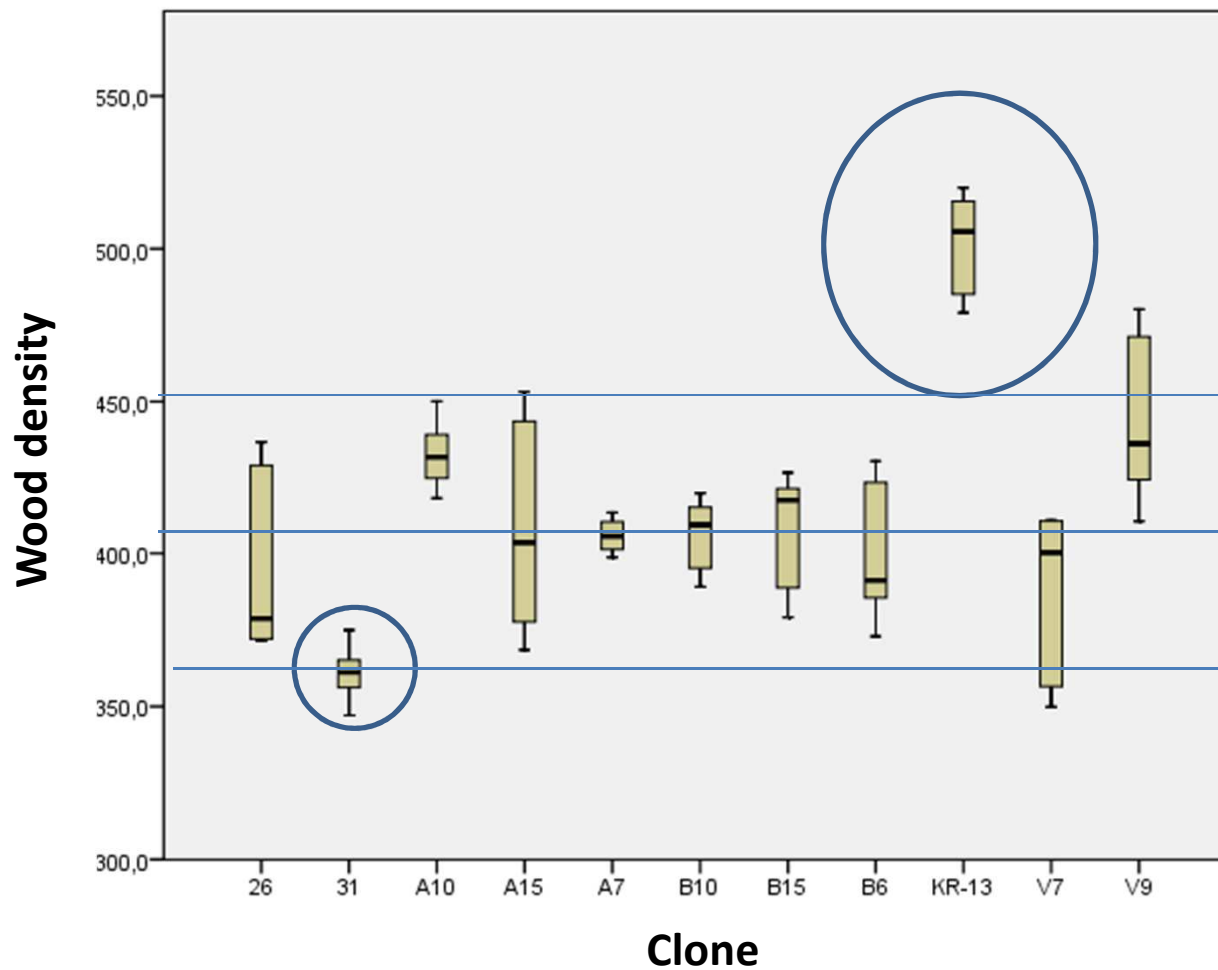
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Results



SILVA





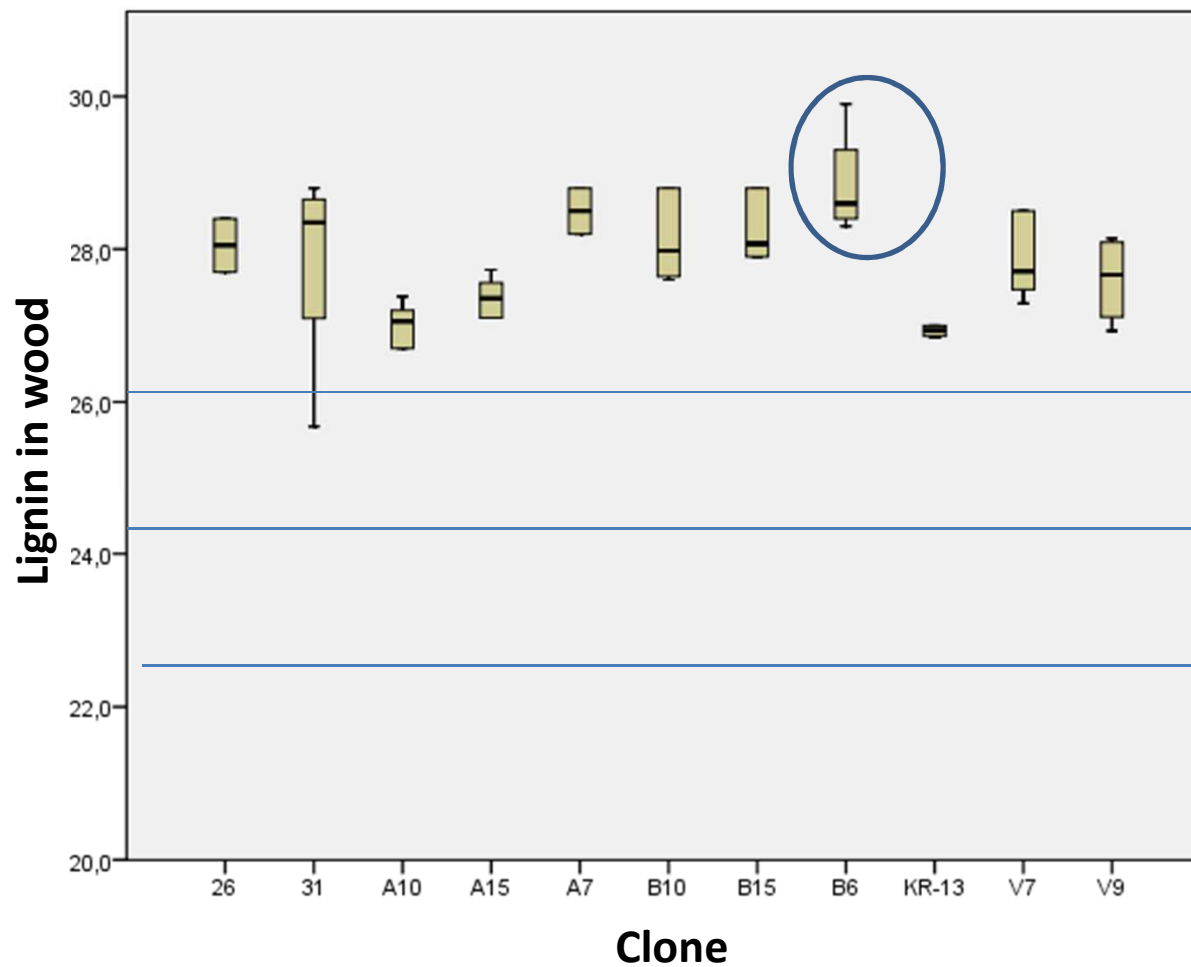
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Results



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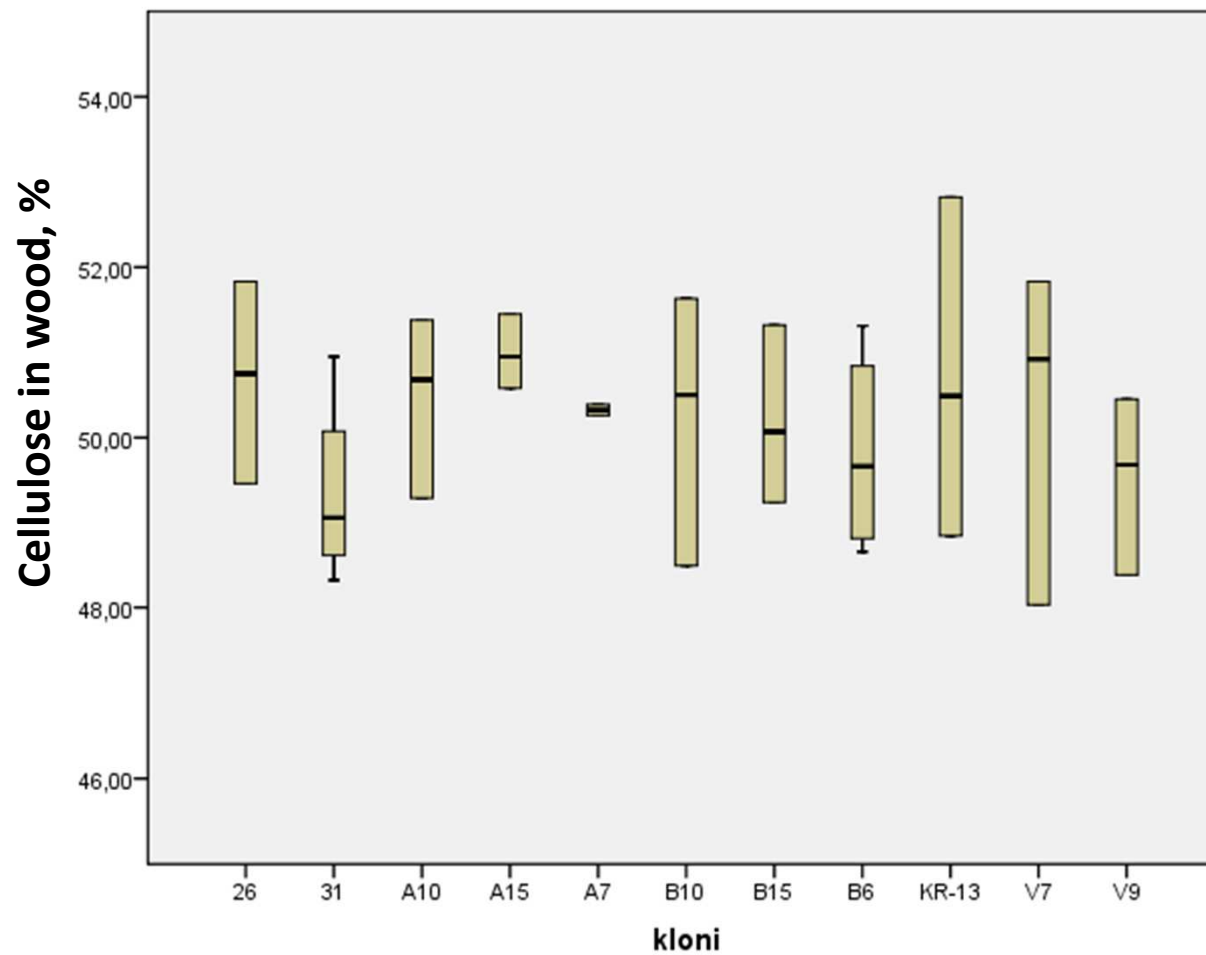
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Results



SILAVA





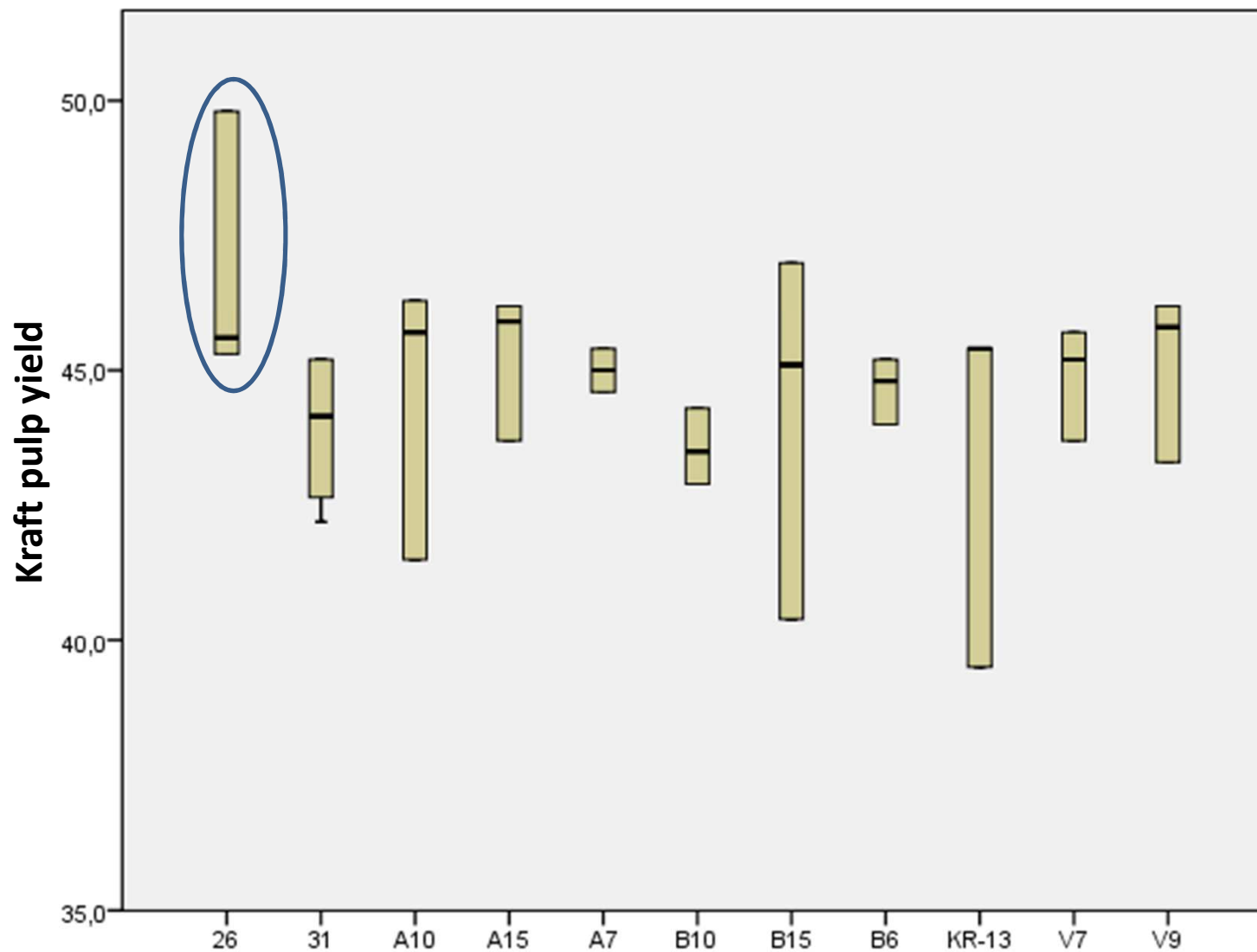
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Results



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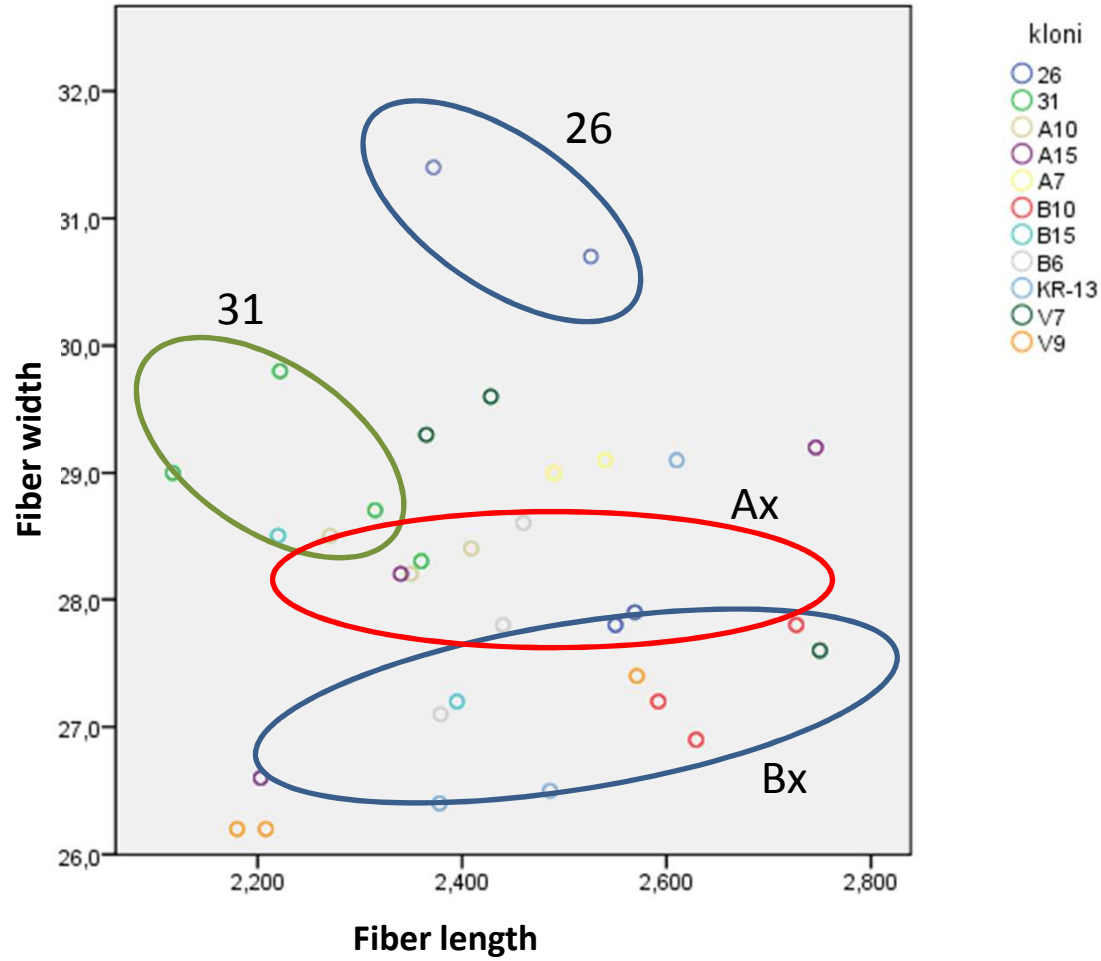
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Results



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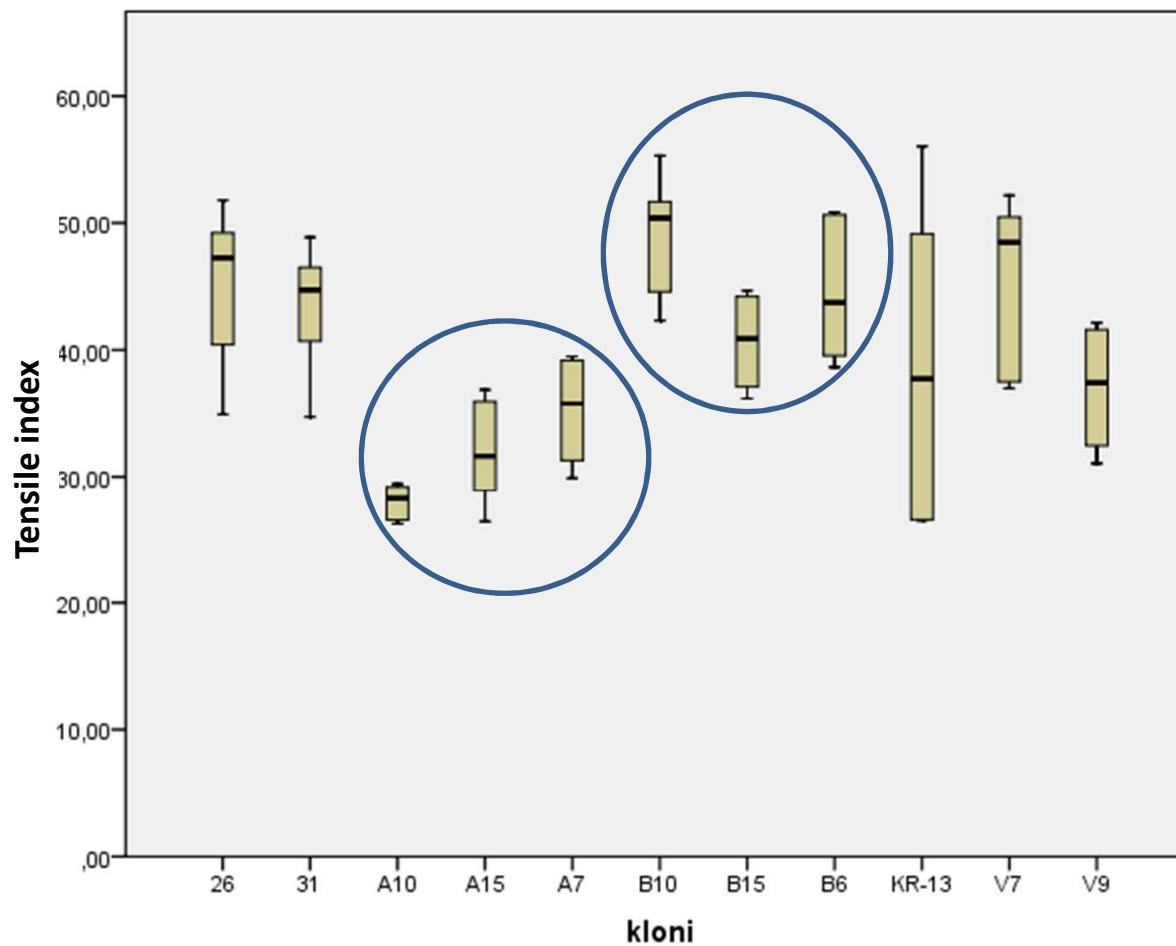
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Results



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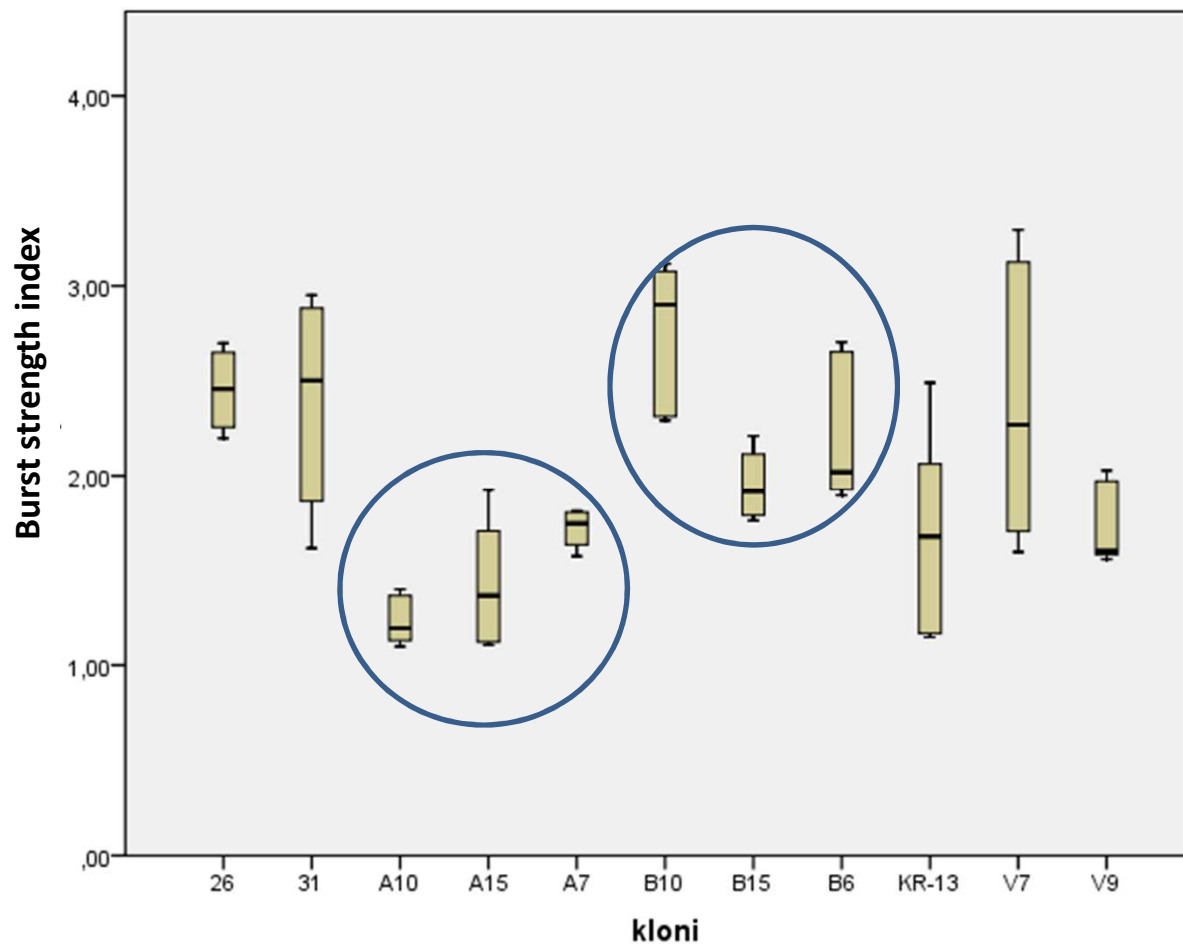
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Results



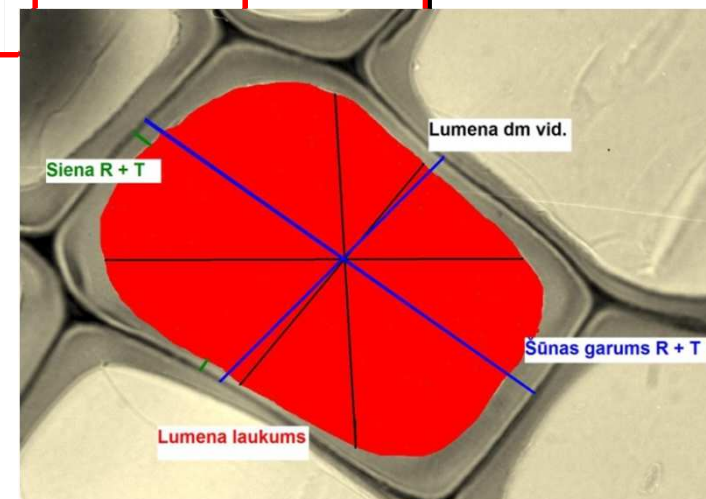
SILAVA



Results

		Fiber length	Fiber width	Runkel l.w.	Runkel e.w.
Tensile index	Pearson Correlation	,185	-,117	-,305	-,645**
	Sig. (2-tailed)	,151	,364	,114	,000
	N	62	62	28	28
Burst index	Pearson Correlation	,150	,037	-,274	-,625**
	Sig. (2-tailed)	,243	,776	,158	,000
	N	62	62	28	28
Breaking length	Pearson Correlation	,203	-,110	-,315	-,650**
	Sig. (2-tailed)	,114	,396	,102	,000
	N	62	62		

Runkel ratio = 2x fiber wall thickness / lumen diameter





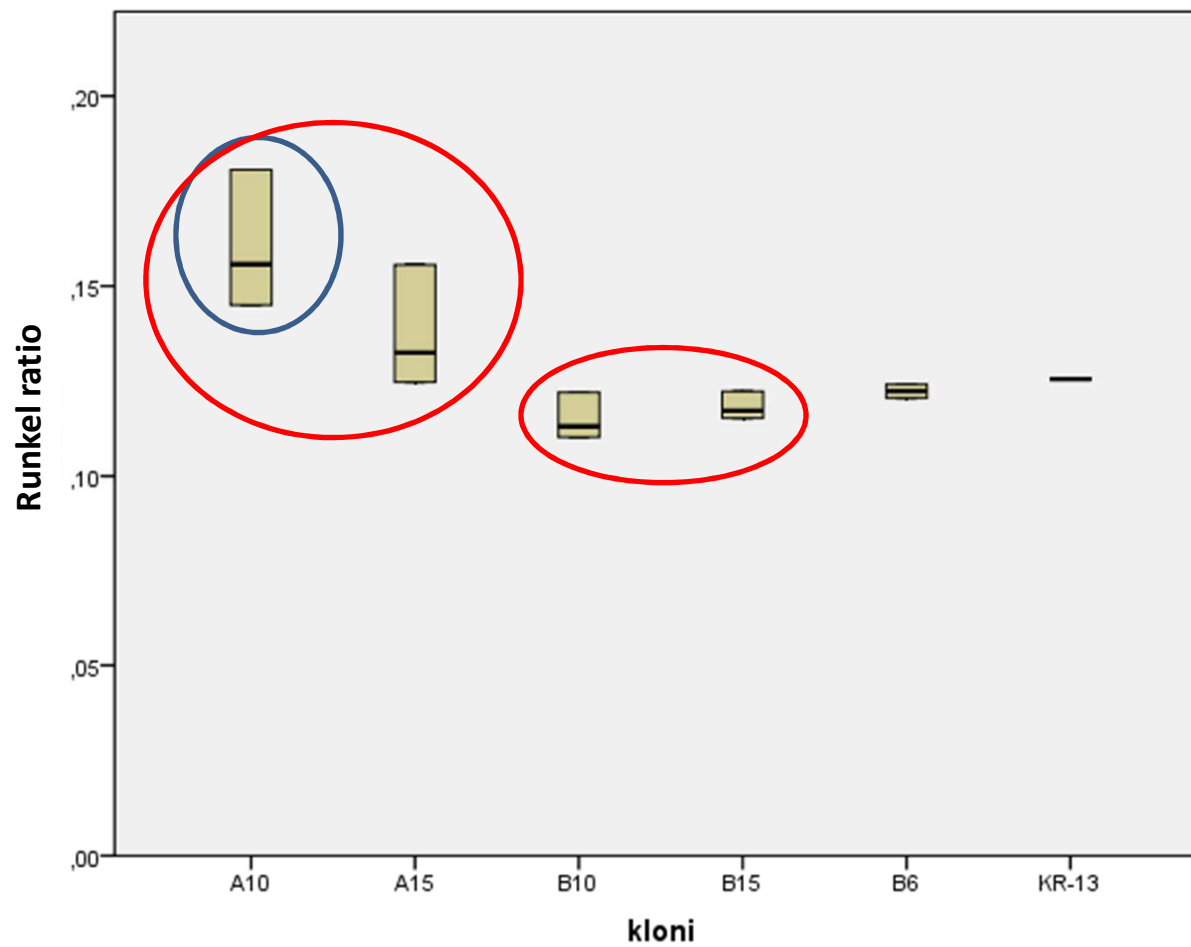
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Results



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Conclusion



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1. More wood produce 26, 31, B10, V7 Sitka spruce clones
2. Kraft pulp yield doesn't differ significant, but more pulp is possible to produce from clone #26.
3. No significant differences found in fiber length, but is found significant differences in fiber width between clones
4. Is significant difference in paper tensile strength between Ax and Bx spruce clones. Bx shows higher results compare to Ax.
5. Fiber morphology microscopy measurments shows, that Runkel ratio in earlywood effect mechanical properties than fiber latewood.



**Study has been partly funded by:
ESF project “Importance of Genetic Factors in Formation of
Forest Stands with High Adaptability and Qualitative Wood
Properties”
(No 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146)**



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Genetic determination of wood traits in Scots pine - analysis of candidate genes influencing wood quality

Krista Kānberga–Siliņa

Latvian State Forest Research Institute
«Silava»

Salaspils 2011

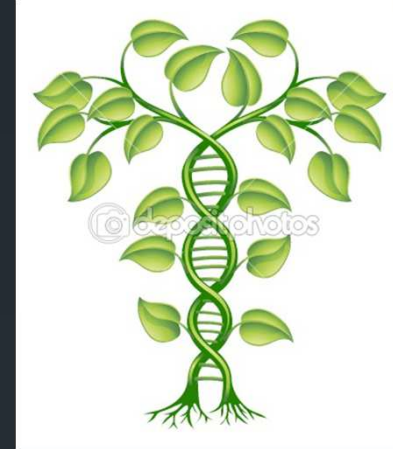
Introduction



- With a few exceptions, little is known about the cellular, molecular, and developmental processes that underlie wood formation.
- Wood formation is controlled by exogenous (photoperiod and temperature) and endogenous (phytohormones) factors and by interaction between them.
- Wood (secondary xylem) formation four major steps : cell division, cell expansion (elongation and radial enlargement), cell wall thickening (involving cellulose, hemicellulose, cell wall proteins, and lignin biosynthesis and deposition) and programmed cell death.
- Wood density is one of main characteristics of wood quality.



Genetic aspects



- Wood formation is driven by the coordinated expression of numerous structural (some of known function) and regulatory genes (mostly unknown).
- The very large genomes of all *Pinus* species, long life cycle, large multigenic families and high metabolic elasticity are some of the reasons why pines were less studied at molecular level.
- Now there are linkage maps, EST databases and gene expression data available for *P.taeda* and *P.radiata* so comparative genomics approach can be used for information transfer to other important pine species.
- Gene copy number variation (CNV) is type of genetic variation. In human genomes CNV affects gene expression. In *Arabidopsis thaliana* and *Sorghum bicolor* gene duplications altered ion transport.



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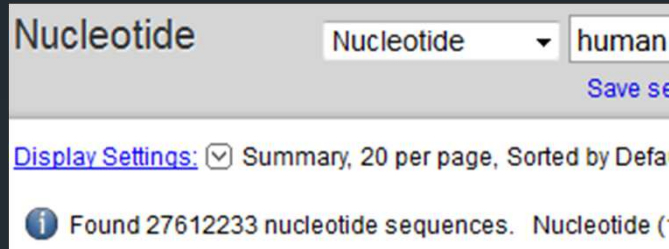
Aim

- To find out if it is possible to find link between wood density and genetic parameters in Scots pine
- For future dream- marker assisted selection for Scots pine.
- Taking in consideration one genetic variation types –CNV.



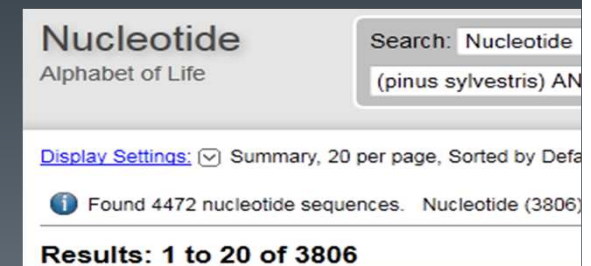
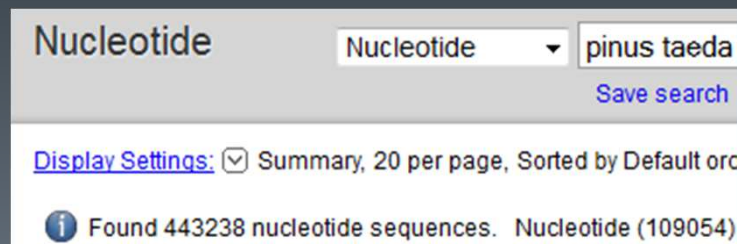
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Methods

- Wood density measured with Pilodyn instrument.
- Selected 25 trees with higher wood density and 25 with lower, belonging to different tree families.
- Literature analysis and searching in databases for candidate genes.
- Sample gathering (Spring and Autumn), DNA, RNA extracting, purification, PCR, Real time PCR optimization.
- Quantitative RT PCR for detecting gene copy number variation.
- Relative standard curve RT PCR for gene expression analysis.
- Data analysis



Candidate genes

- At first nine genes were taken in consideration (*CAD1*, *CESA1*, *CESA7*, *CESA4*, *AGP1*, *aquaporin like*, *aquaporin gene*, *Susy1*).

- Candidate genes were selected of which three are involved in lignin biosynthesis – phenylalanine ammonia-lyase gene (*PAL1*), cinnamyl alcohol dehydrogenase gene (*CAD1*), Cinnamoyl-CoA reductase gene (*CCR1*);

sucrose synthase gene (*Susy1*) in sucrose metabolism ;

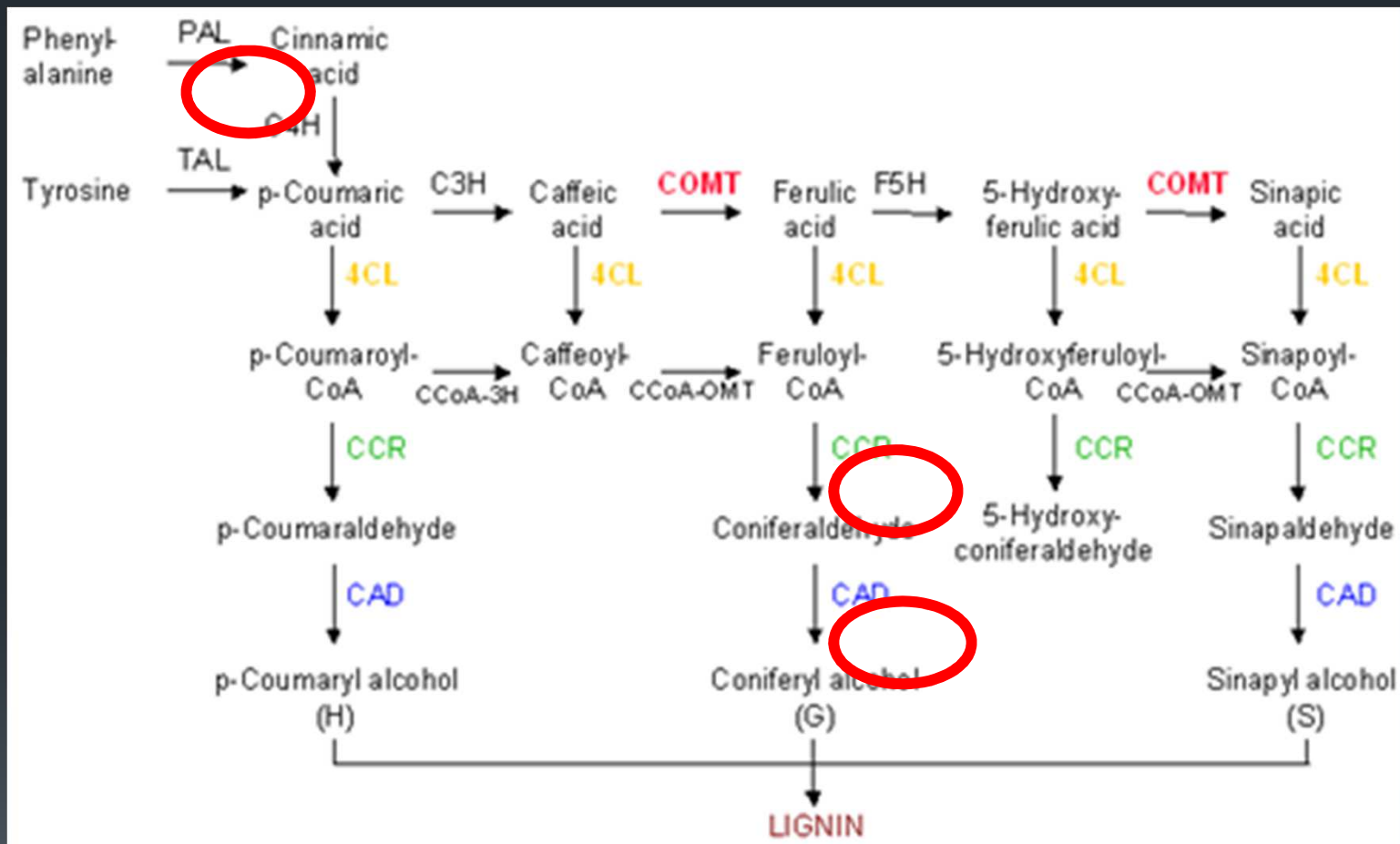
Aquaporin like gene (*Aqual1*) involved in water transport, in attempts to reveal whether link can be established between candidate gene expression, gene copy number and wood density .



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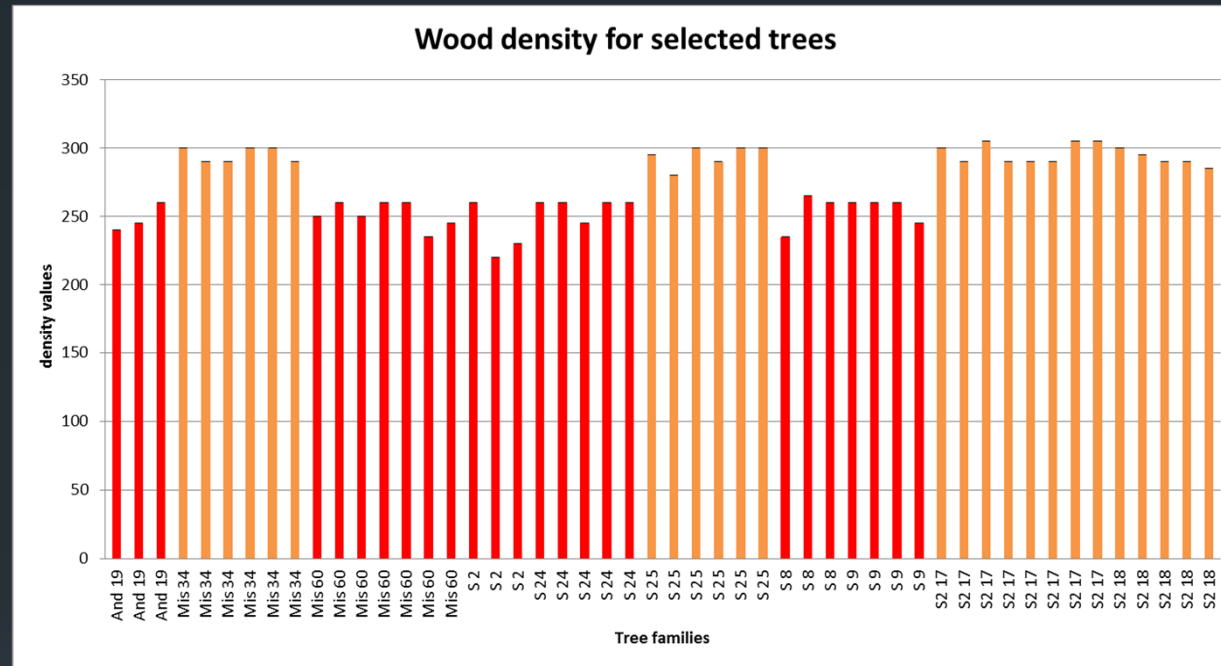
Lignin biosynthesis



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Wood density



Orange - trees with higher wood density

Red - with lower wood density

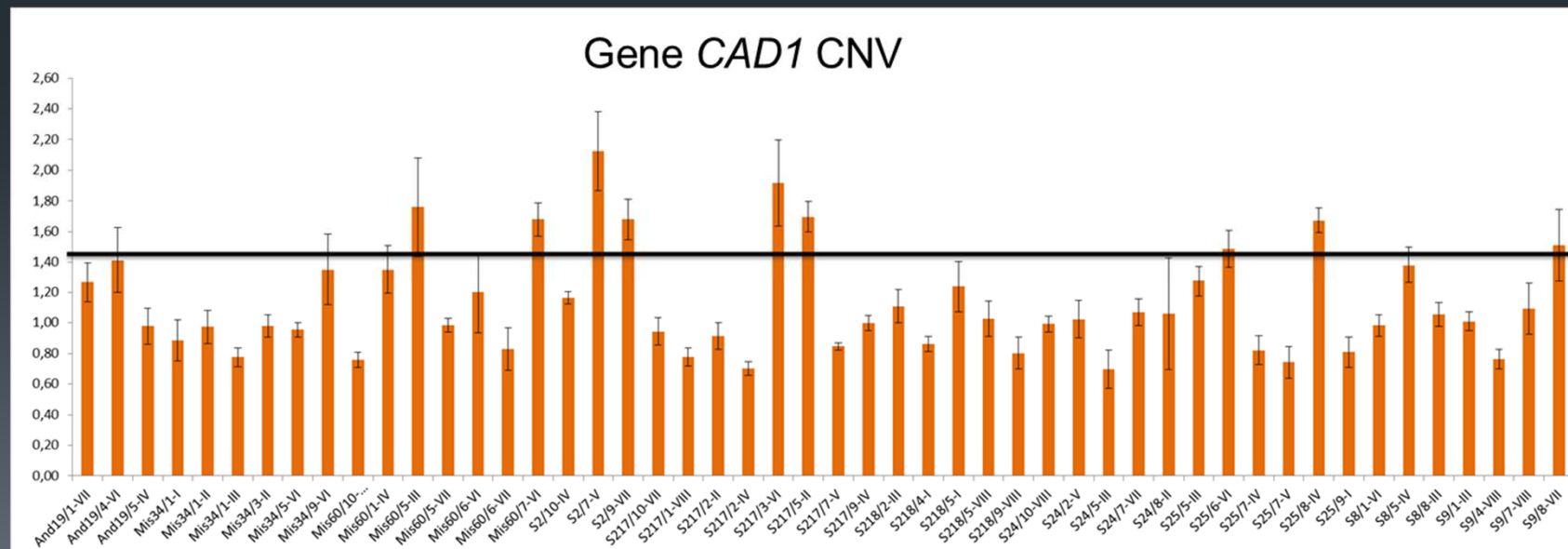
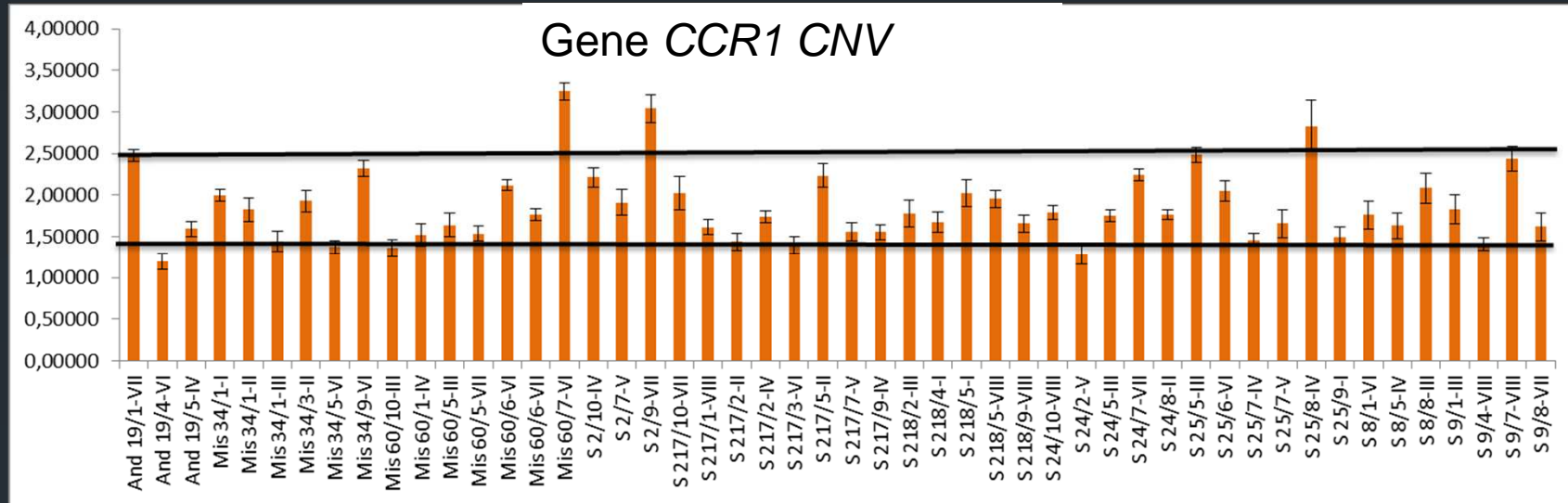


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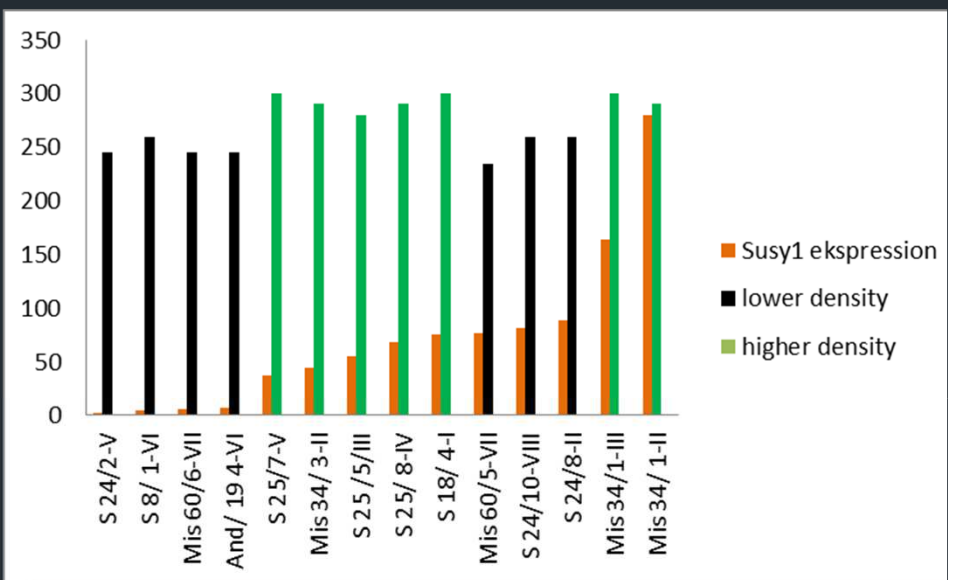
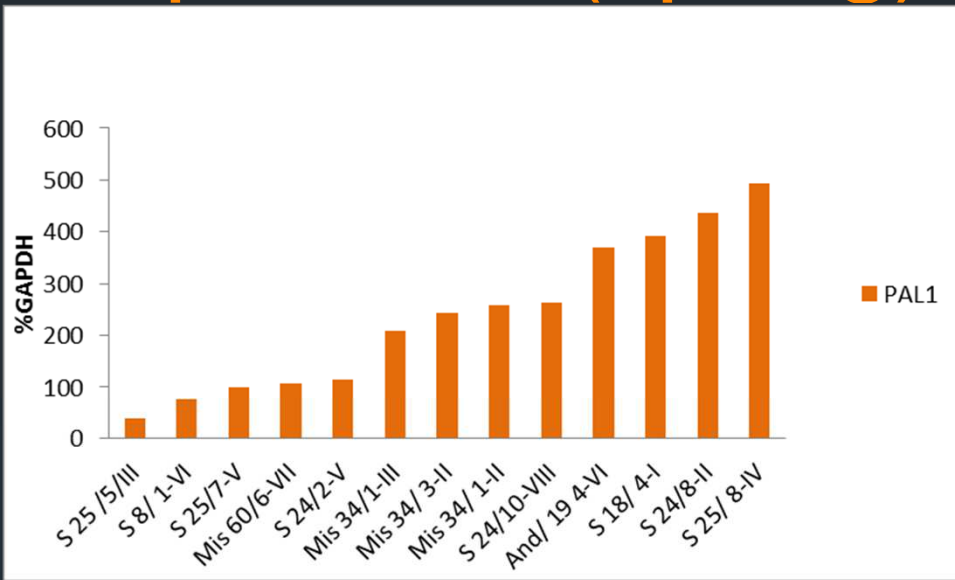
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Copy number variation of gene *CCR1* and *CAD1*

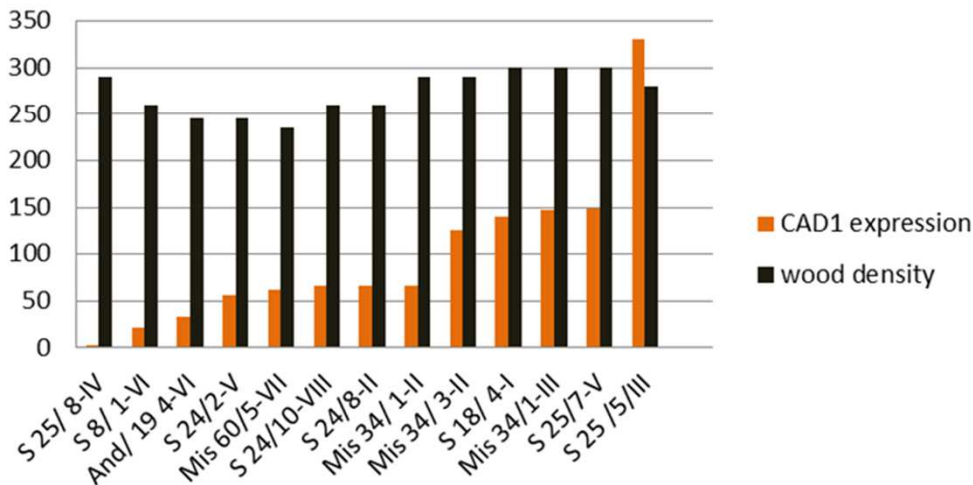
$|Z| < 2$



Gene *PAL1*, *CAD1*, *Susy1* expression (spring)

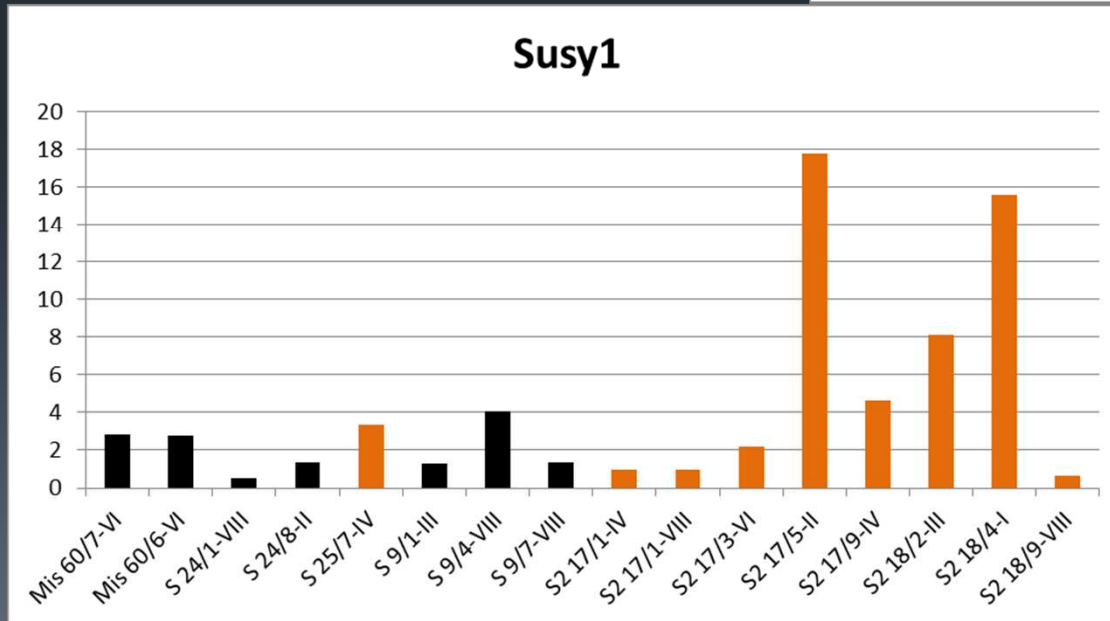
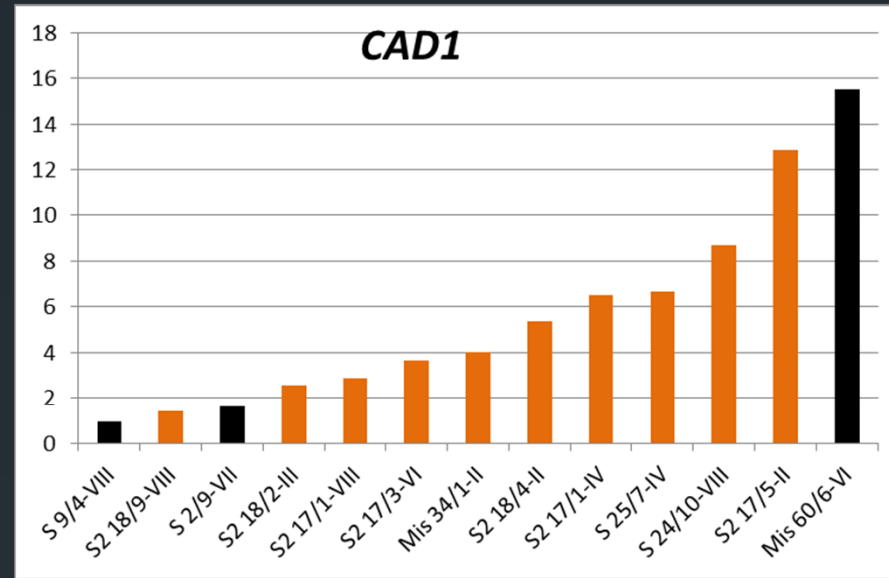


CAD1



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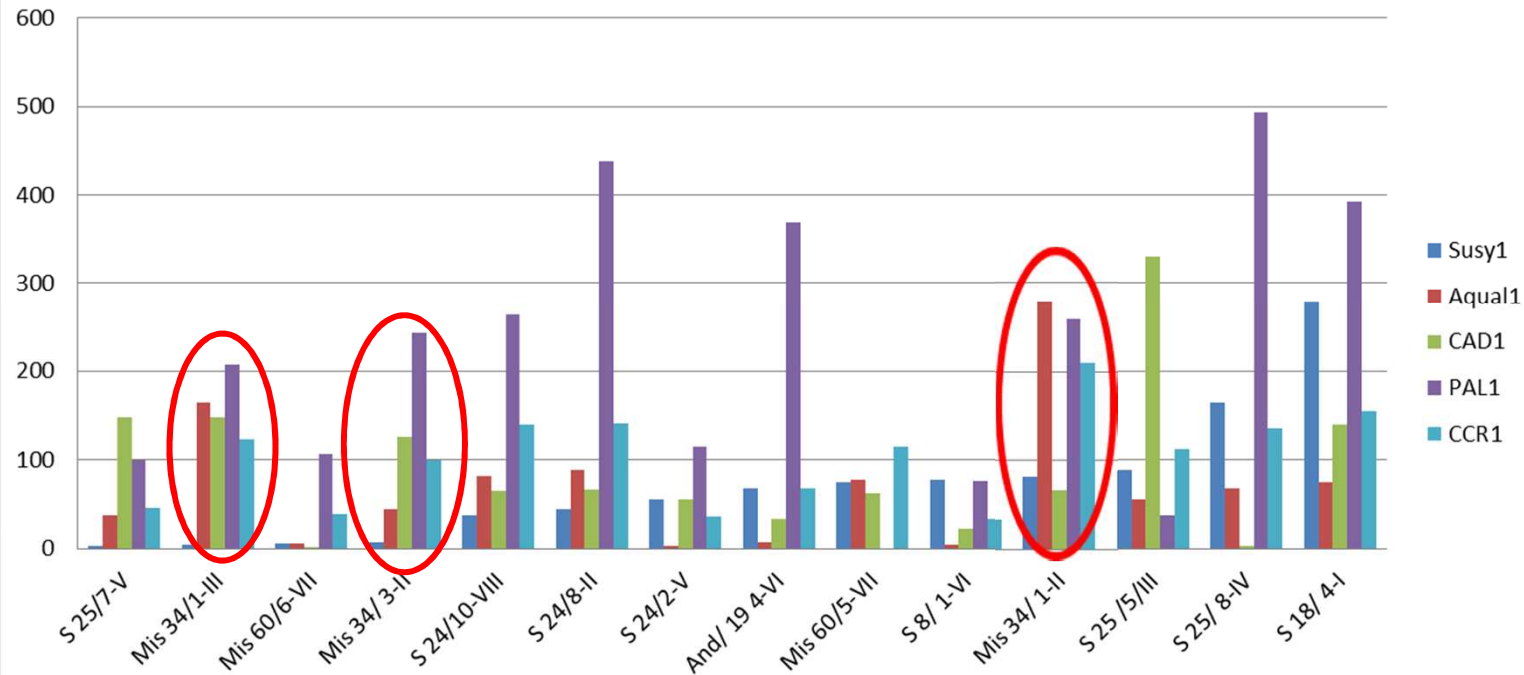
Autumn



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Gene expression comparison for spring samples



PAL1 ekspression higher 2x than for others
Mis 34 perspective

	<i>Susy1</i>	<i>Aqual1</i>	<i>CAD1</i>	<i>PAL1</i>	<i>CCR1</i>
<i>Susy1</i>	1				
<i>Aqual1</i>	0,063774	1			
<i>CAD1</i>	0,043073	0,11905	1		
<i>PAL1</i>	0,4313	0,205608	-0,3006	1	
<i>CCR1</i>	0,400035	0,838829	0,166369	0,492318	1



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Some conclusions

- Quantitative RT PCR can be used to determine gene copy number variation for *P.sylvestris*.
- CNV data confirms suggestions about genes PAL1 and CAD1- that there are single functional gene copy in pines.
- There are positive regression between gene *SuSy1* and *CAD1* expression in spring and wood density, so we can assume that early wood density is affected significantly by carbohydrate availability and last step of monolignol biosynthesis .
- Between genes *CCR1* and *Aqual1* exists strong correlation for spring samples, which may indicate about gene interaction in wood formation process.



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Future research plans

- Continue gene expression determination for genes *CAD1*, *PAL1*, *Aqual1*, *Susy1*, *CCR1* for all samples and possibly more.
- Analyse more candidate genes, for example transcription factors *MYB1*, *MYB2*. Search for genes involved in monolignol transportation (coniferyl alcohol glucosyltransferase).
- Microscopic evaluation of cellulose/lignin content, cell wall thickness. Or at least earlywood/latewood proportion measurement.
- Sequencing in hope to find gene regions suitable for marker development



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Conferences and result presentations

- 69. Annual Scientific Conference of University of Latvia, Plant Biology section, 22 february, 2011 (Oral Presentation).
- Scientific conference «Advances in plant biotechnology in Baltic Sea Region» , 30-31 March, 2011, Lithuania, Abstract book p.53-54, (Poster) .
- 2011. Master degree in Biological Sciences in University of Latvia.



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Research funding

European Social Fund's Project "Importance of Genetic Factors in Formation of Forest Stands with High Adaptability and Qualitative Wood Properties"
(No 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146)





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Thank you for attention!



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Genetic diversity with aspen stands: phenology observations and molecular analysis

Mārtiņš Zeps

martins.zeps@silava.lv



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Background



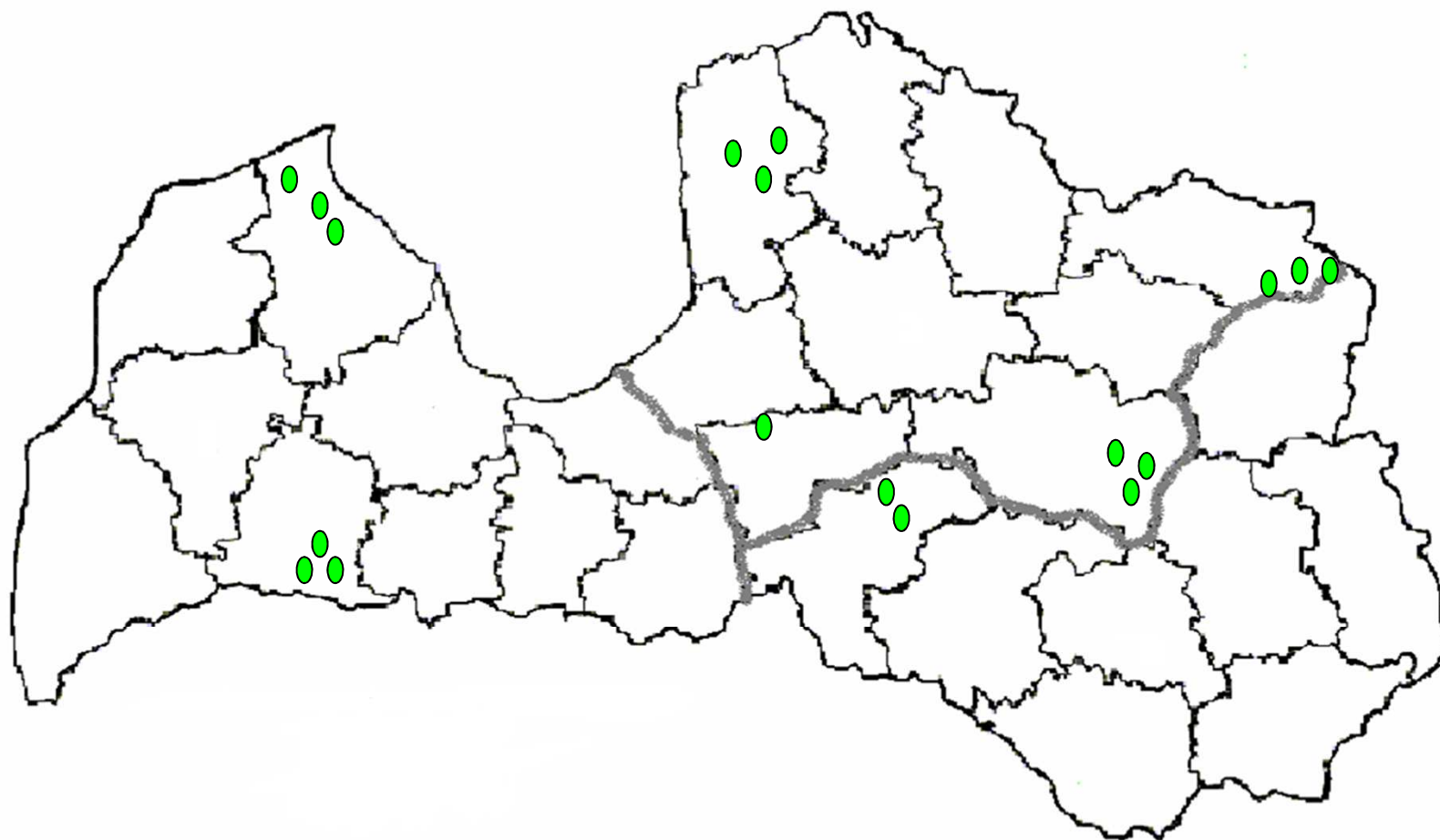
The European aspen (*Populus tremula*) is thought to reproduce mostly asexually. Thus aspen forms clones, in which several ramets belong to one genetically defined genet. We compared the clonal structure of aspen in managed forests in 6 populations in Latvia. In every population selected 3 stands approximately 5 to 10 years old, naturally regenerated this sprouts. Clones were identified using morphological characters and 3+3 microsatellite loci originally developed for *Populus remuloides*.



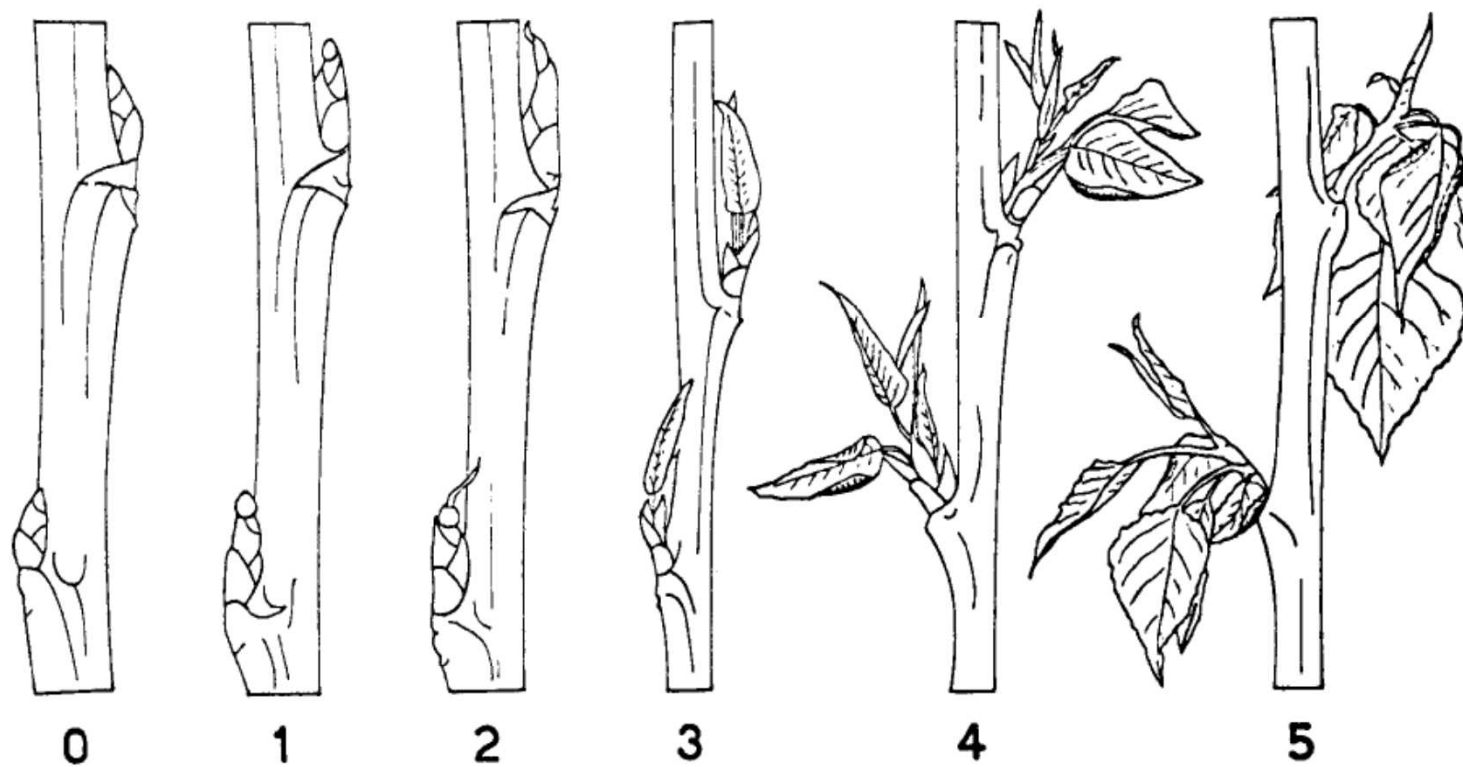
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Materials and method



Materials and method

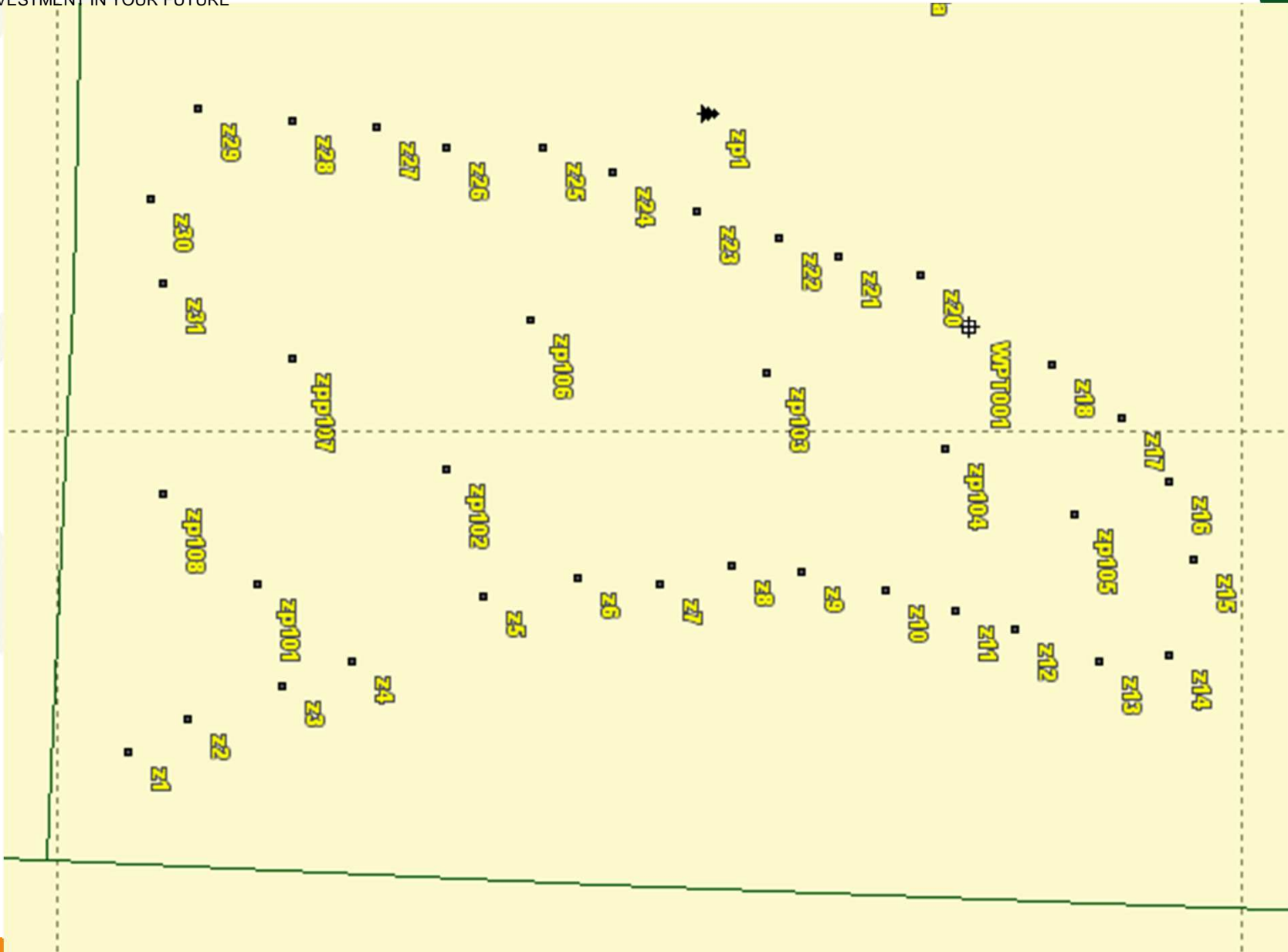




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Results



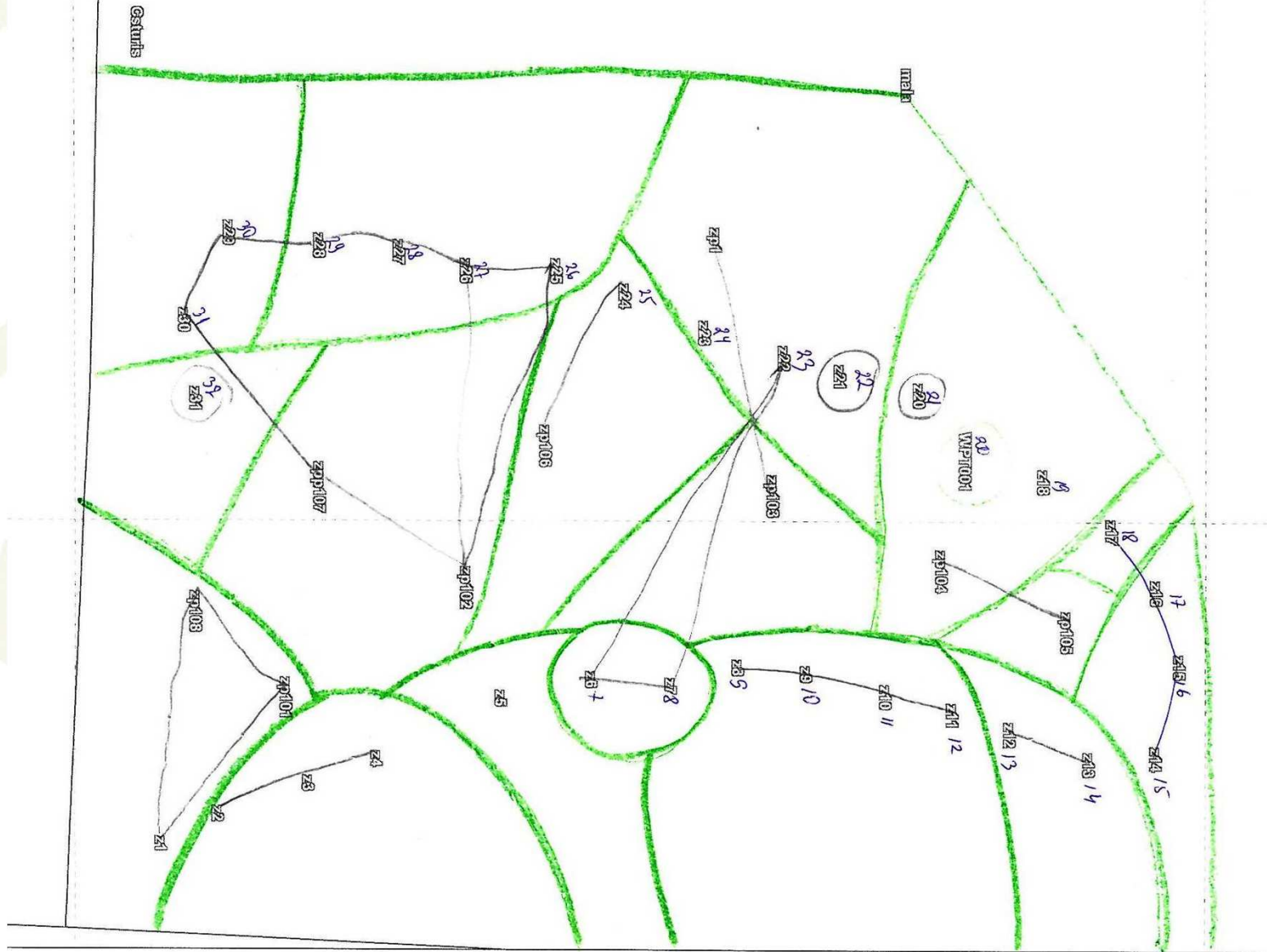


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Results



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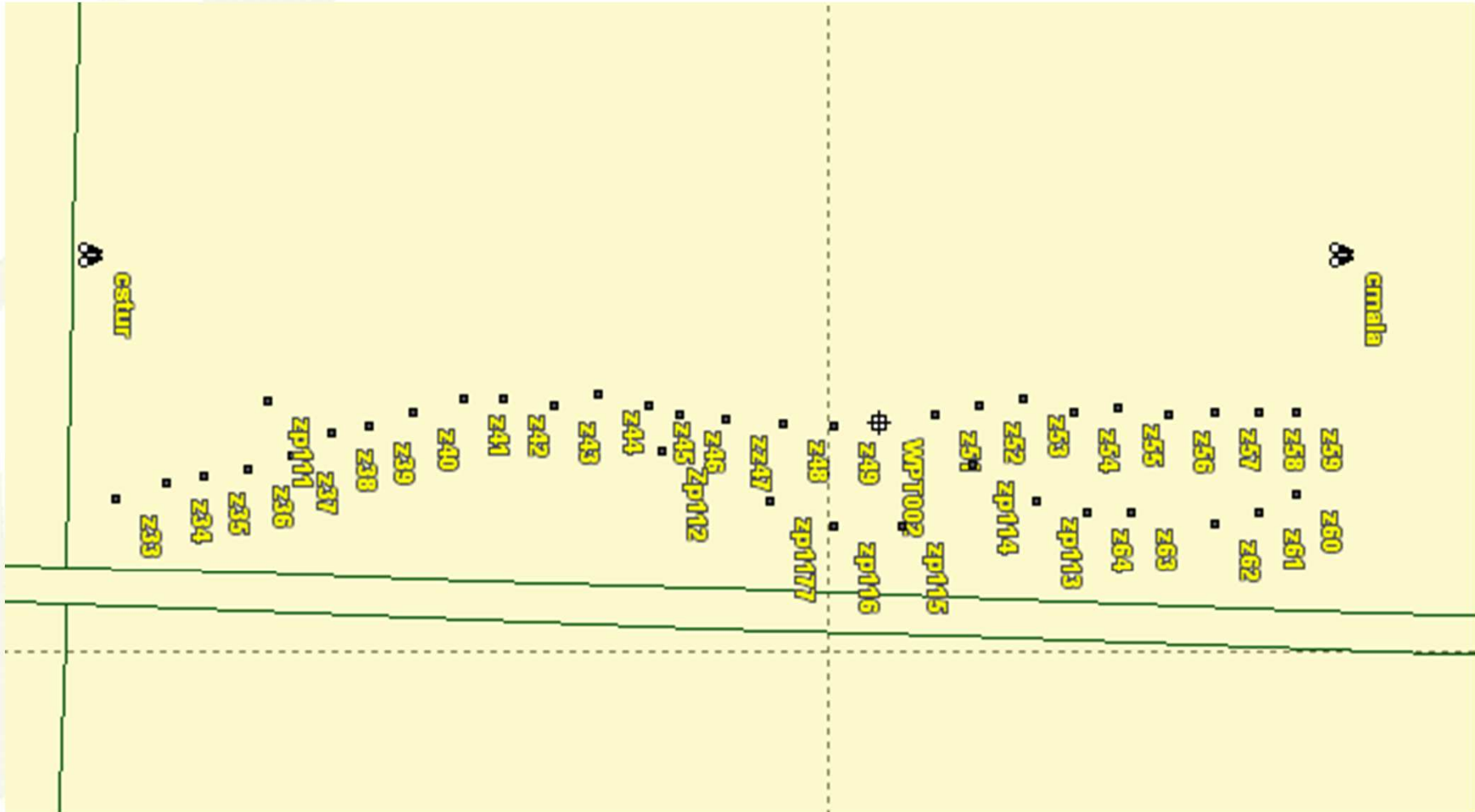




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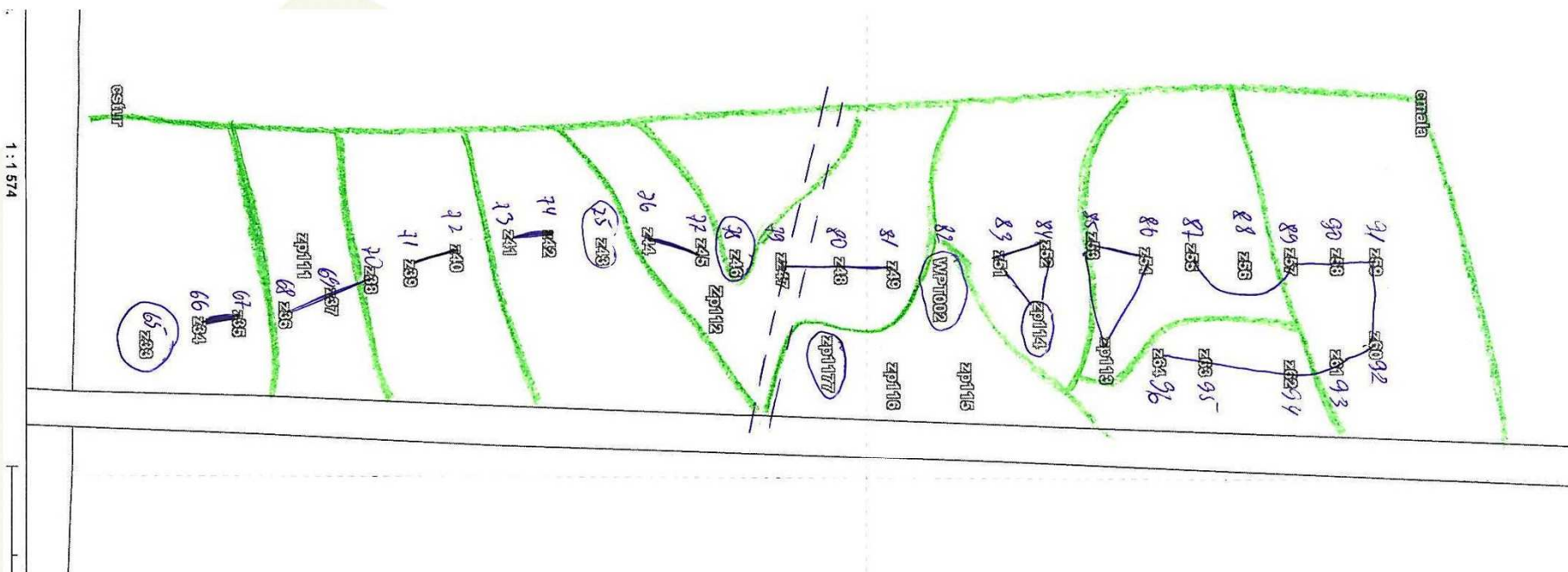
Results



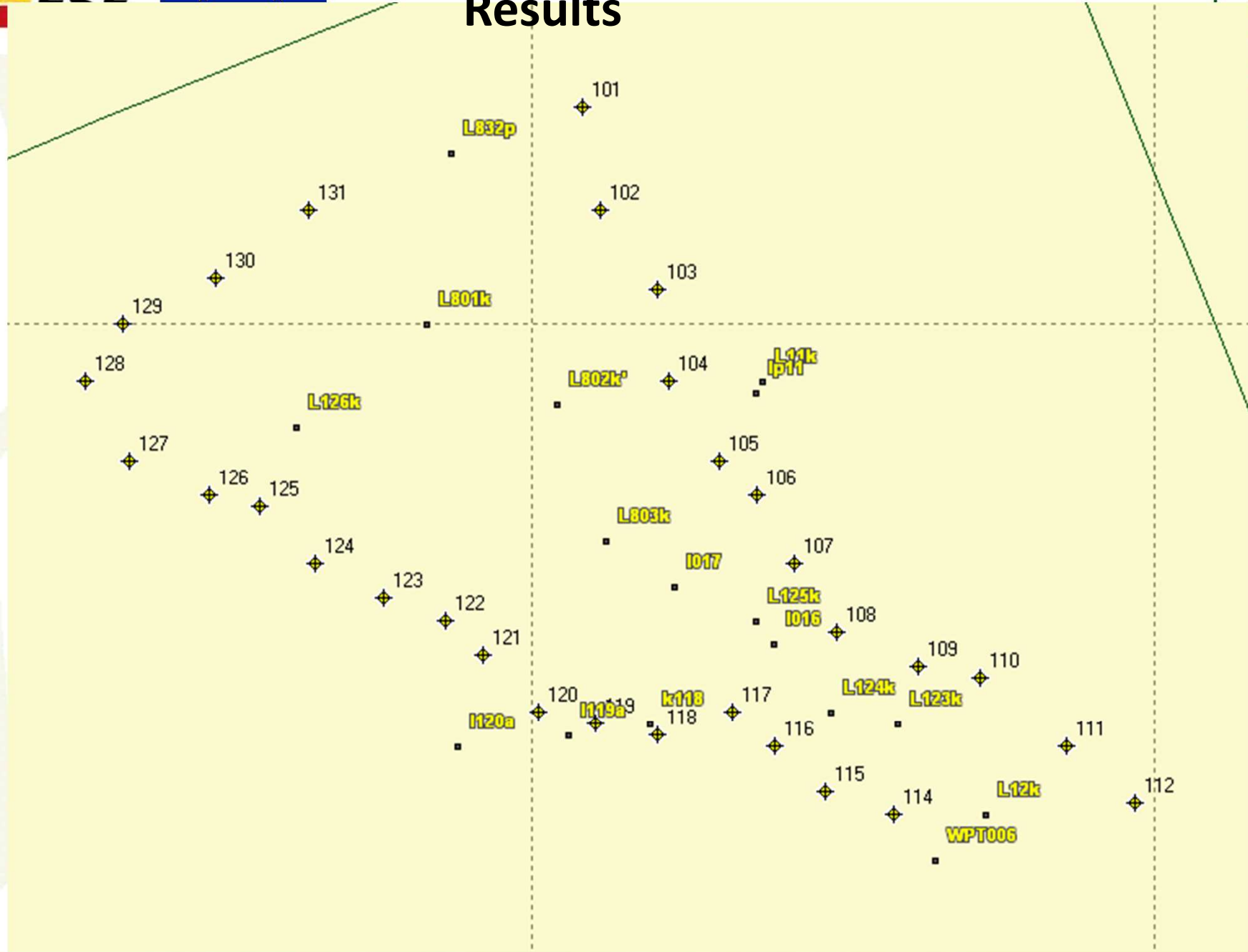


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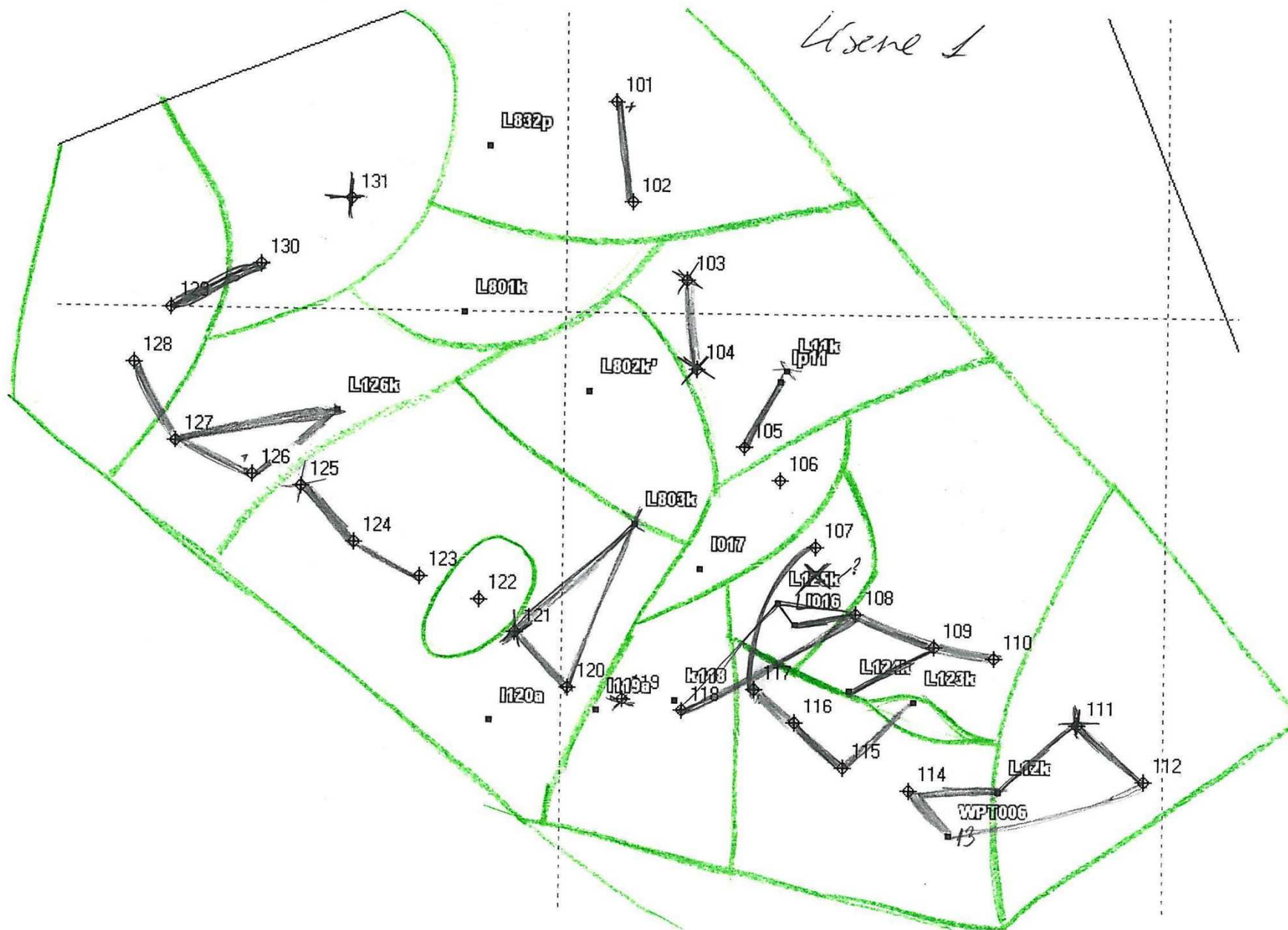


Results





Usene 1





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Conclusions



- ✓ **The numbers of clones that were identified with microsatellites were larger than morphological characters.**
- ✓ **On average clone size in population was 1,6 ramets**
- ✓ **Only one ramets average in population was 22 ramets**
- ✓ **The average number of clones per hectare was 11,3.**



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Thank You for attention!



**Study has been funded by:
ESF project “Importance of Genetic Factors in Formation of Forest
Stands with High Adaptability and Qualitative Wood Properties”
(No 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146)**



Genetic variation in annual height growth of Latvian Norway spruce OP families

Darius Danusevicius, ASU, LT

Aris Jansons, Silava, LV



Objectives

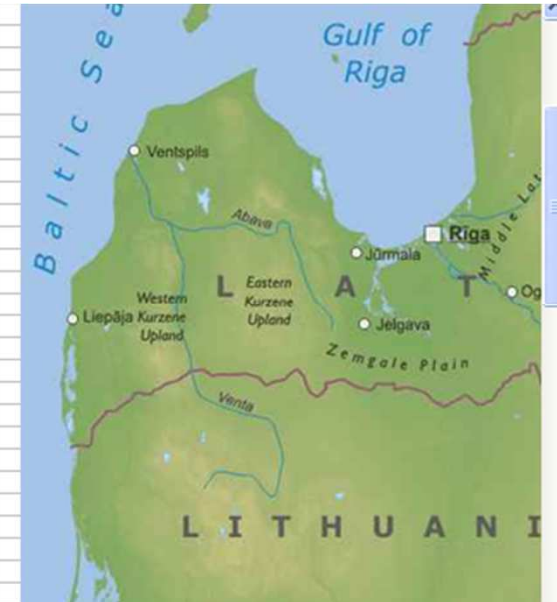
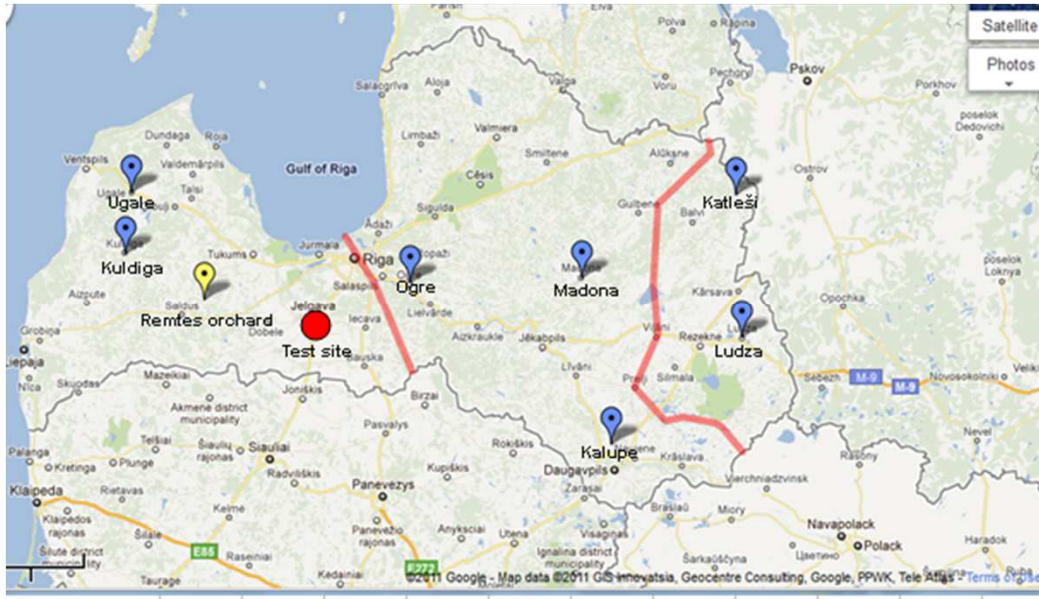
- Assess H growth to reveal adaptive environments and transfer effect within LV
- Patterns of genetic parameters during the growth period



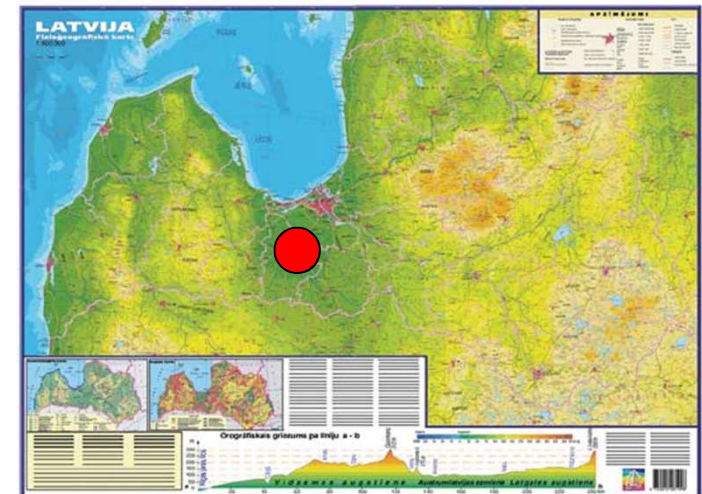
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Material



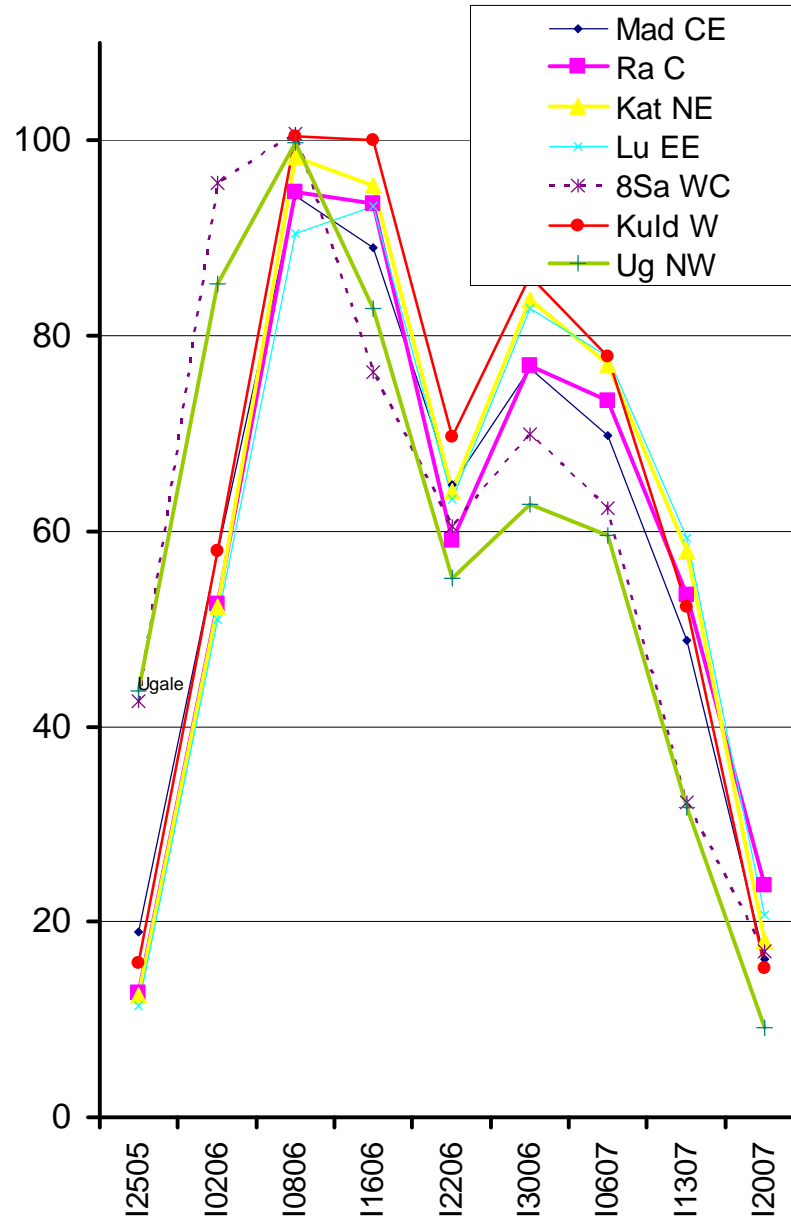
- 56 OP fam.s from 8 pop.s from 3 zones
- All moved from “cooler” to warmer (see altitude)
- 3 pop.s moved from CONT to MARITME





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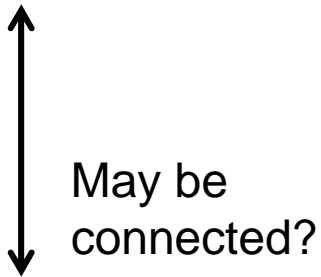




Basic theory

3 major components of shoot length

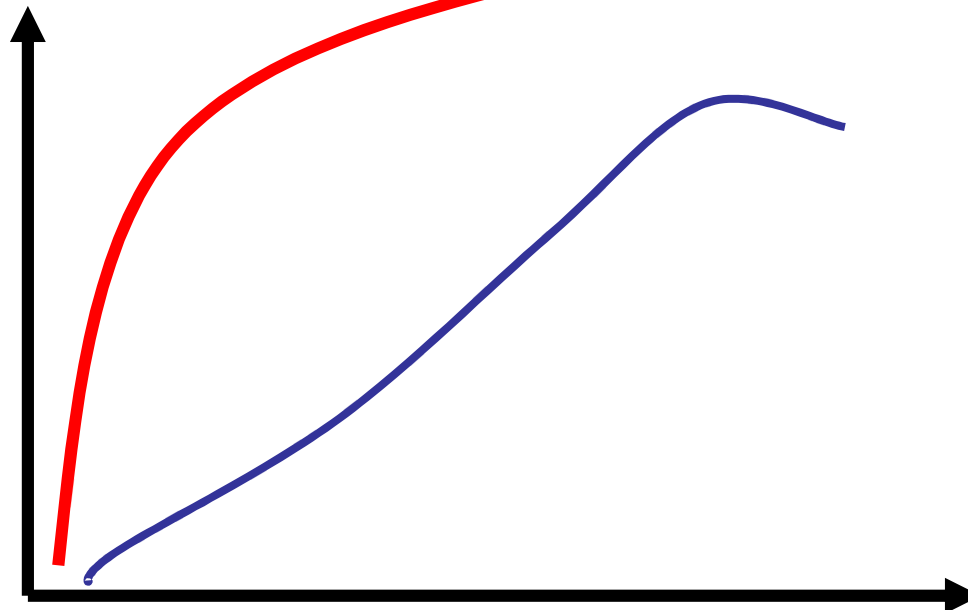
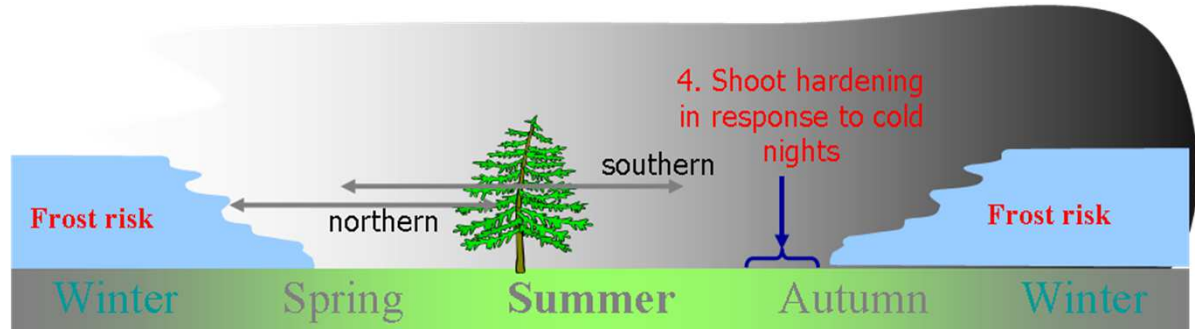
1. Transfer effect = growth rhythm



2. Growth vigor (fam effect)

3. Free growth (sea –continent)

Damage



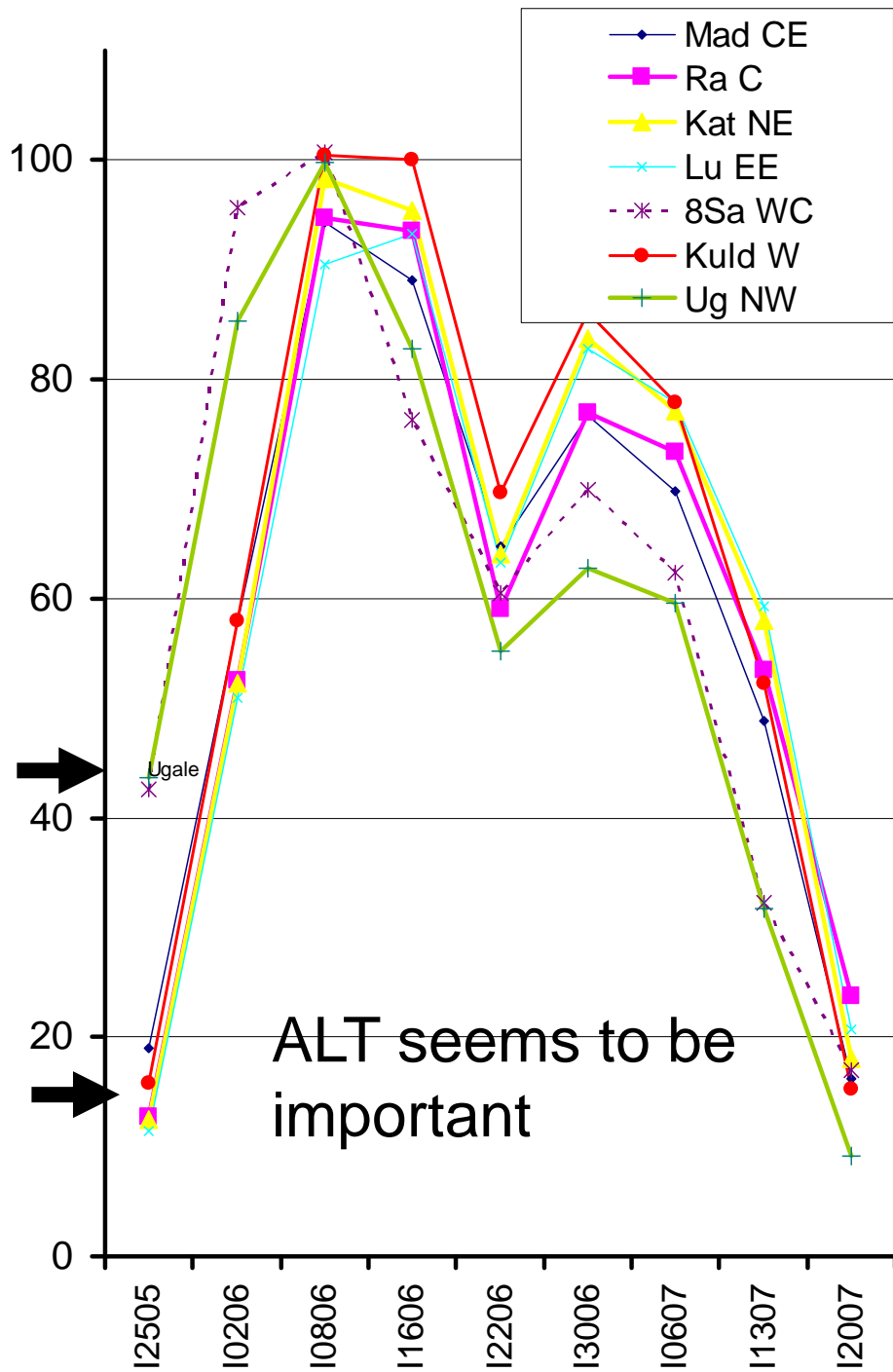
Results

- Population and transfer effect
- Correlations (early start means early end?)
- Genetic parameters and genetic variation at various stages
- Response to stress in June

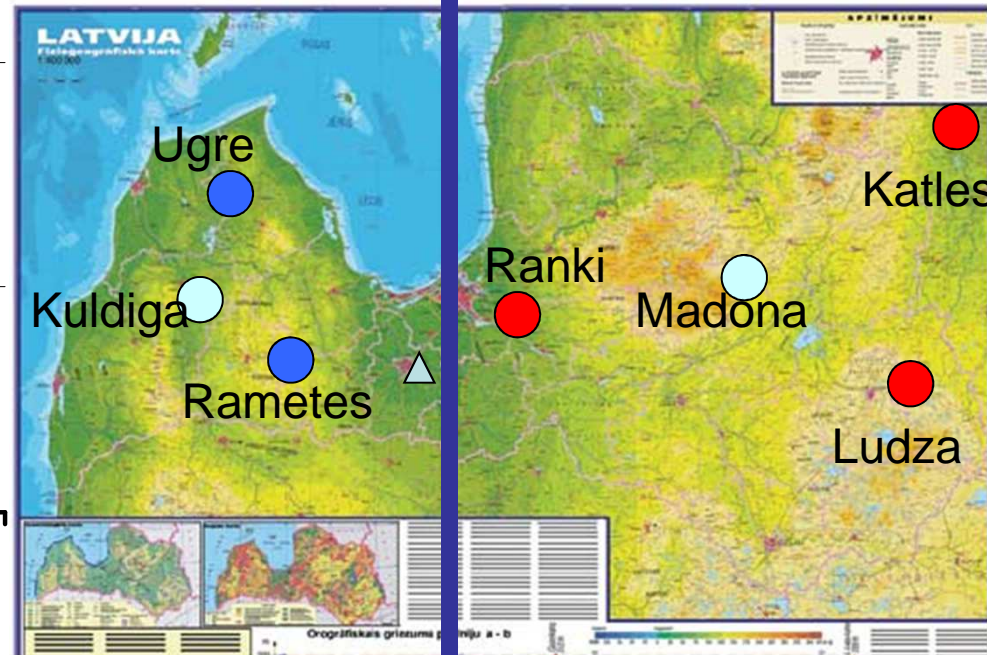
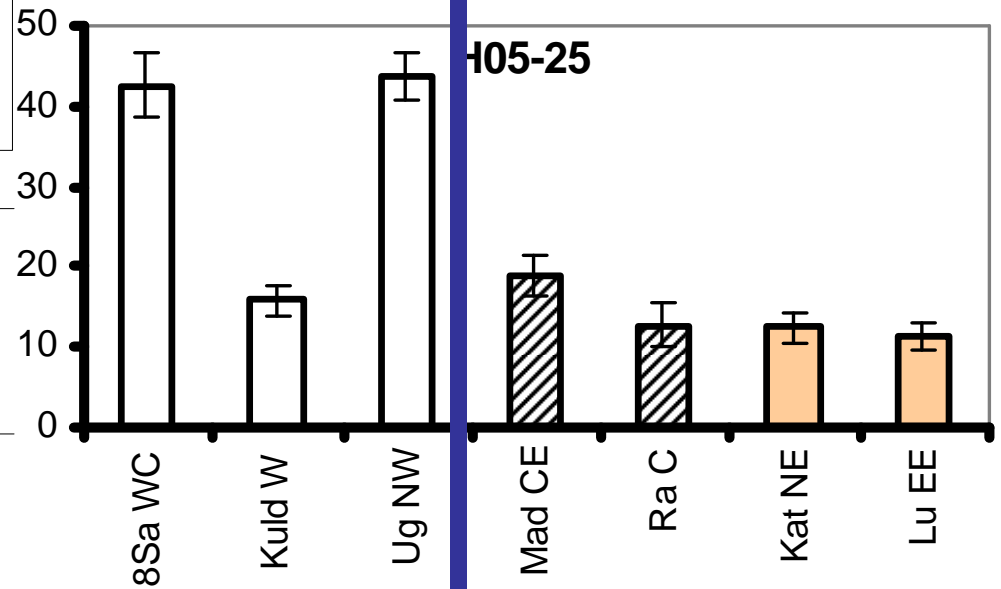
Population (transfer) effect

Bandomuosiuose želdiniuose





Start of GP

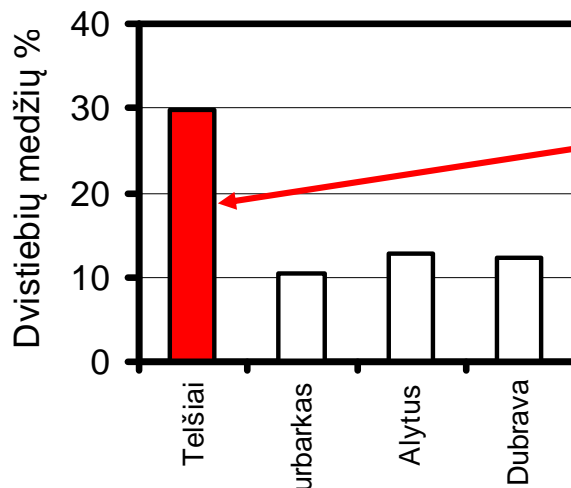
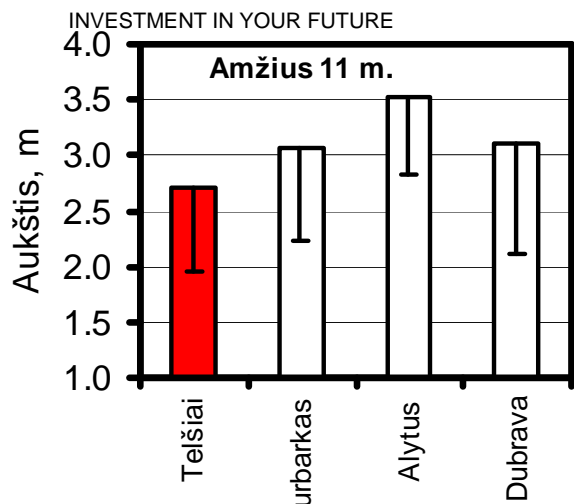


ALT seems to be important

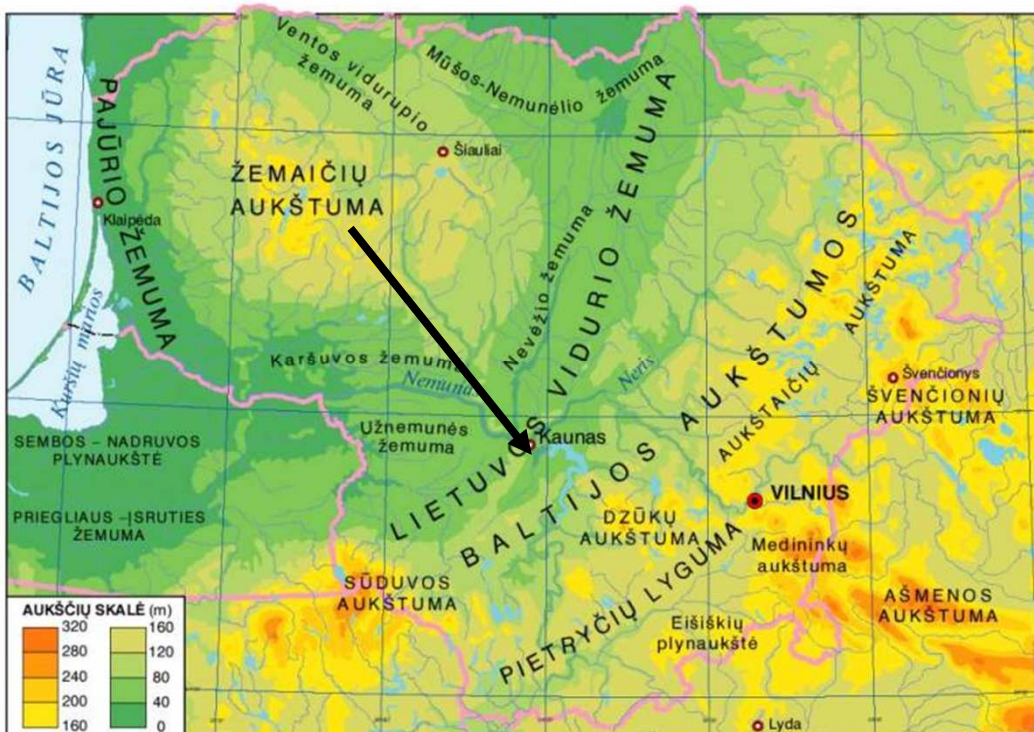


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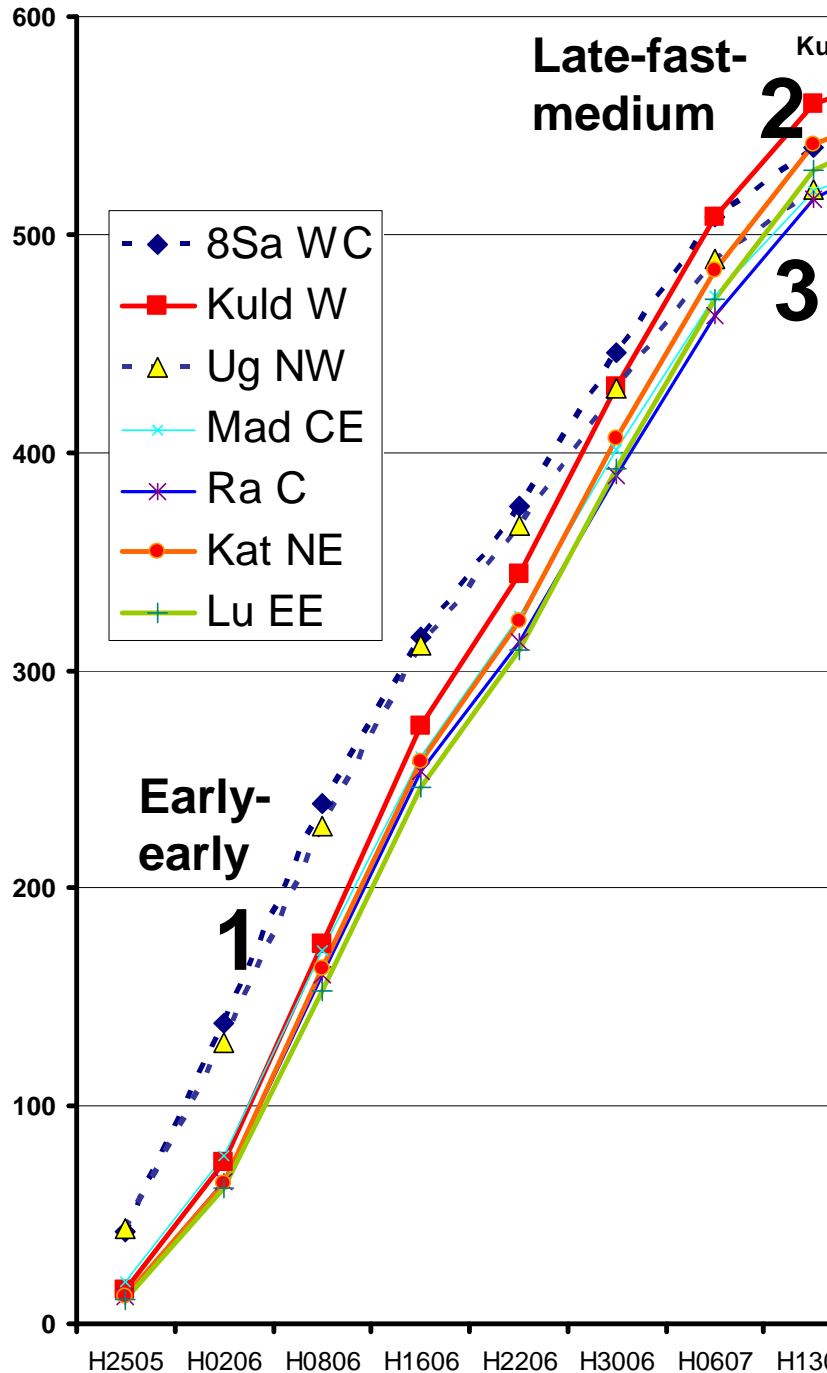
Transfer effects in LT



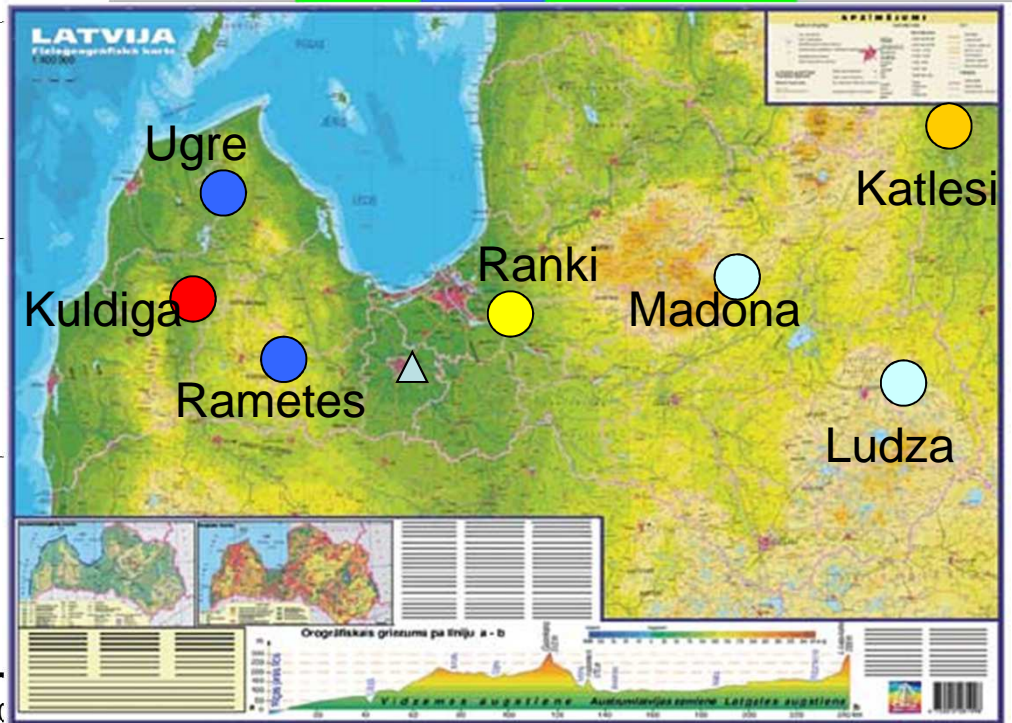
Esminis Lietuvos populiacijų perkėlimo efektas (pvz. iš Telšių į Kauną rajoną)



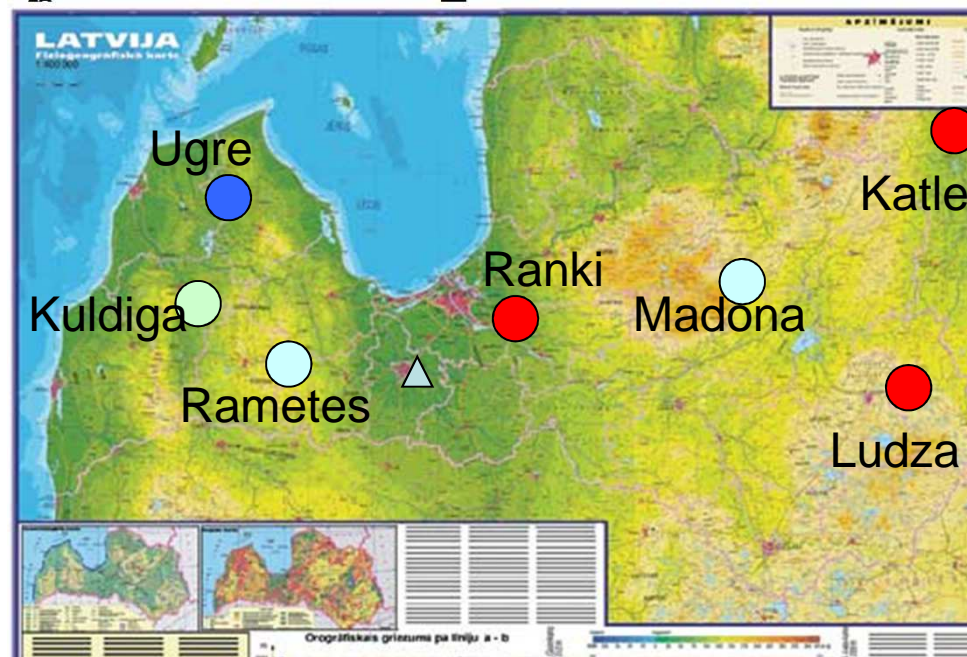
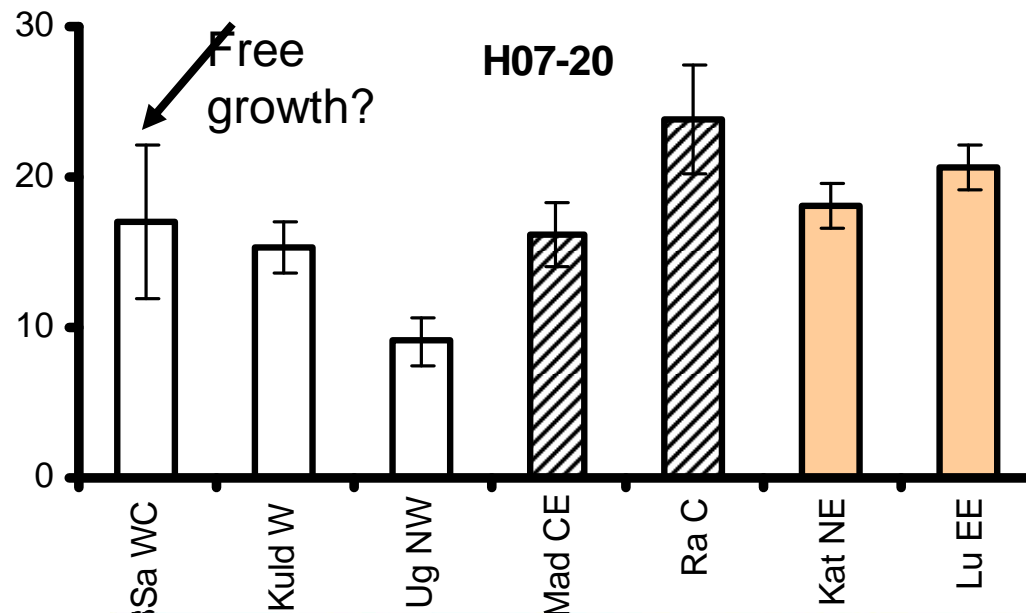
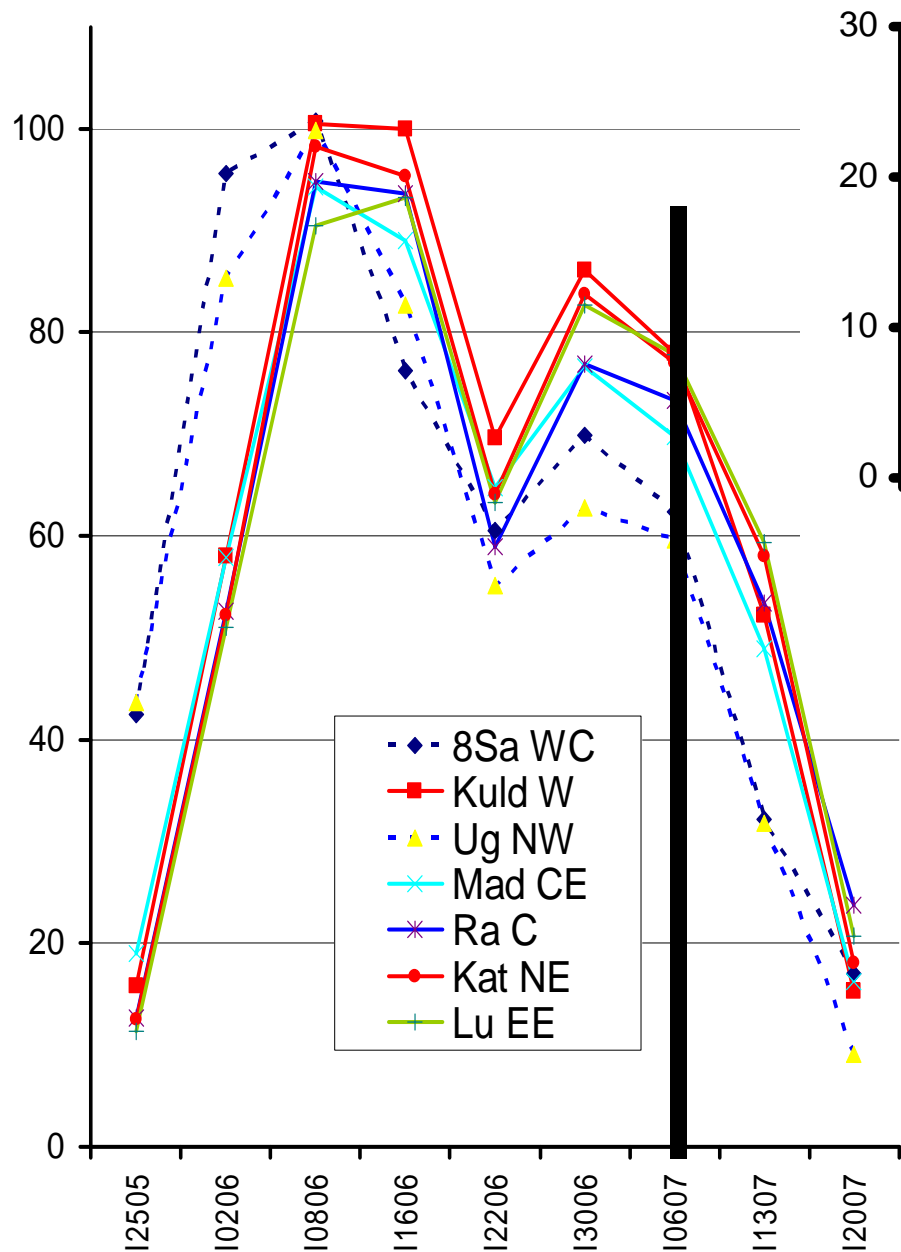
Growth pattern= 4 groups of populations

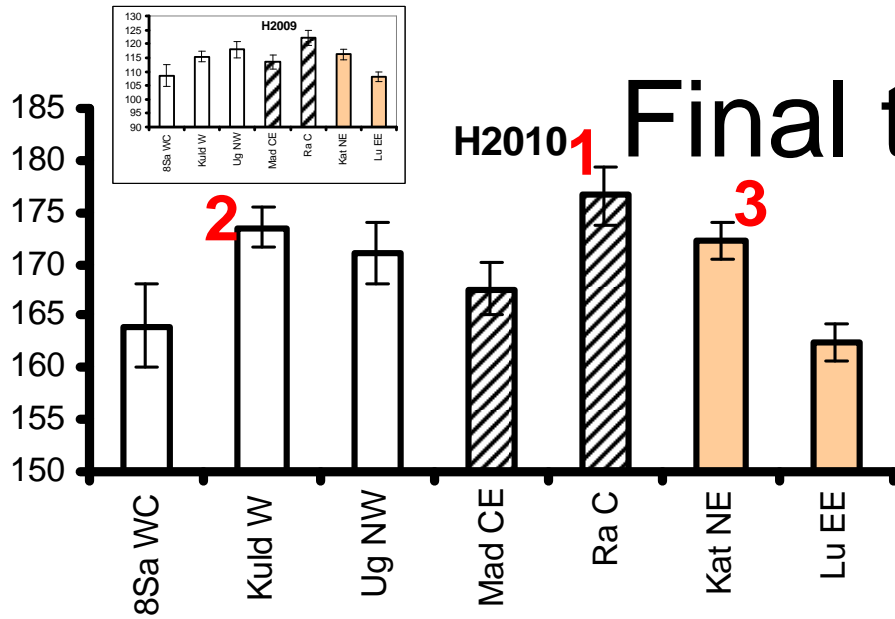


Prov	Zone	Start	Vigjuor Begin	End	Cessation	H rank of 7	
Ugale NW	W	Early	High	Lowest	Earliest	4	Free growth
Ramtes orc	W	Early	High	Low	Early	6	
Kuldiga	W	Late-	High	High	Medium	2	ws 1st rank
Ranki	C	Late	Low	High	Latest	1	Typical sol.
Madona	C	Late-	Medium	Medium	Medium	5	
Katliesi	E	Late	Low	High	Late	3	most cotine
Ludza	E	Late	Low	Medium	Late	7	most cotine

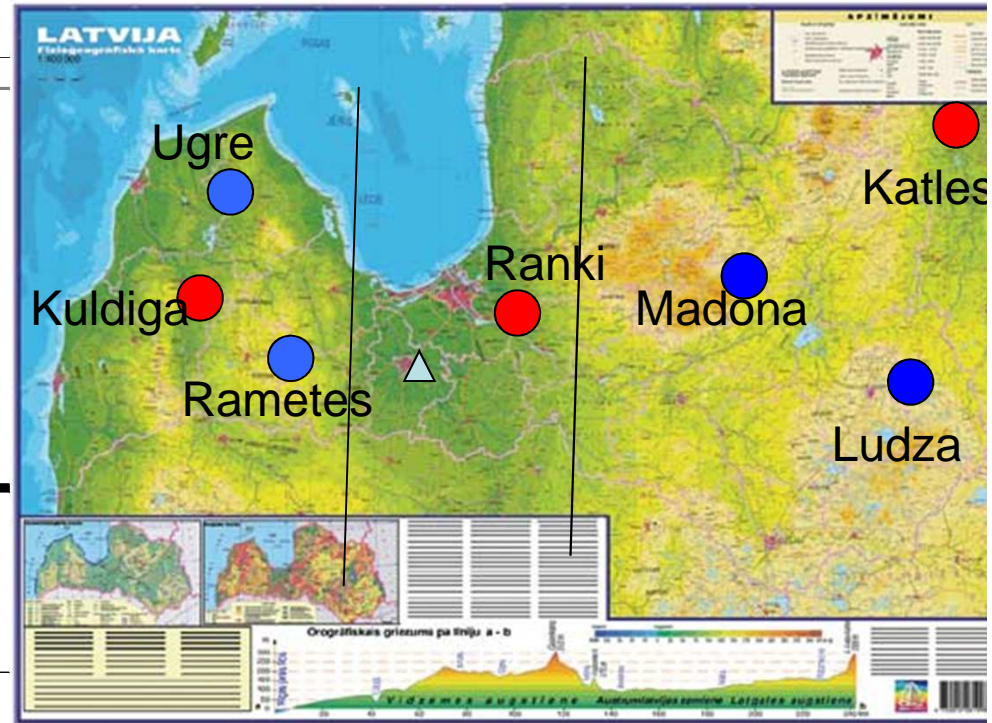
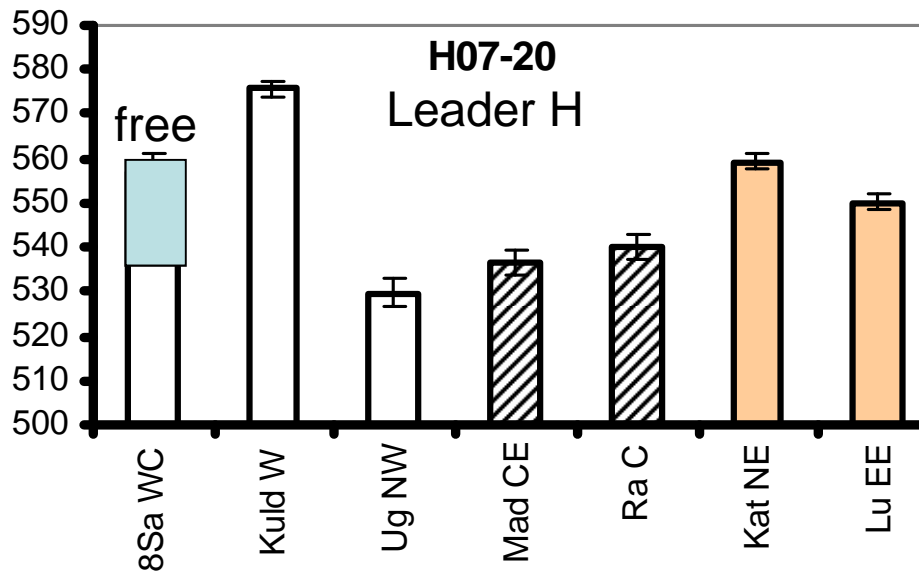


Cessation

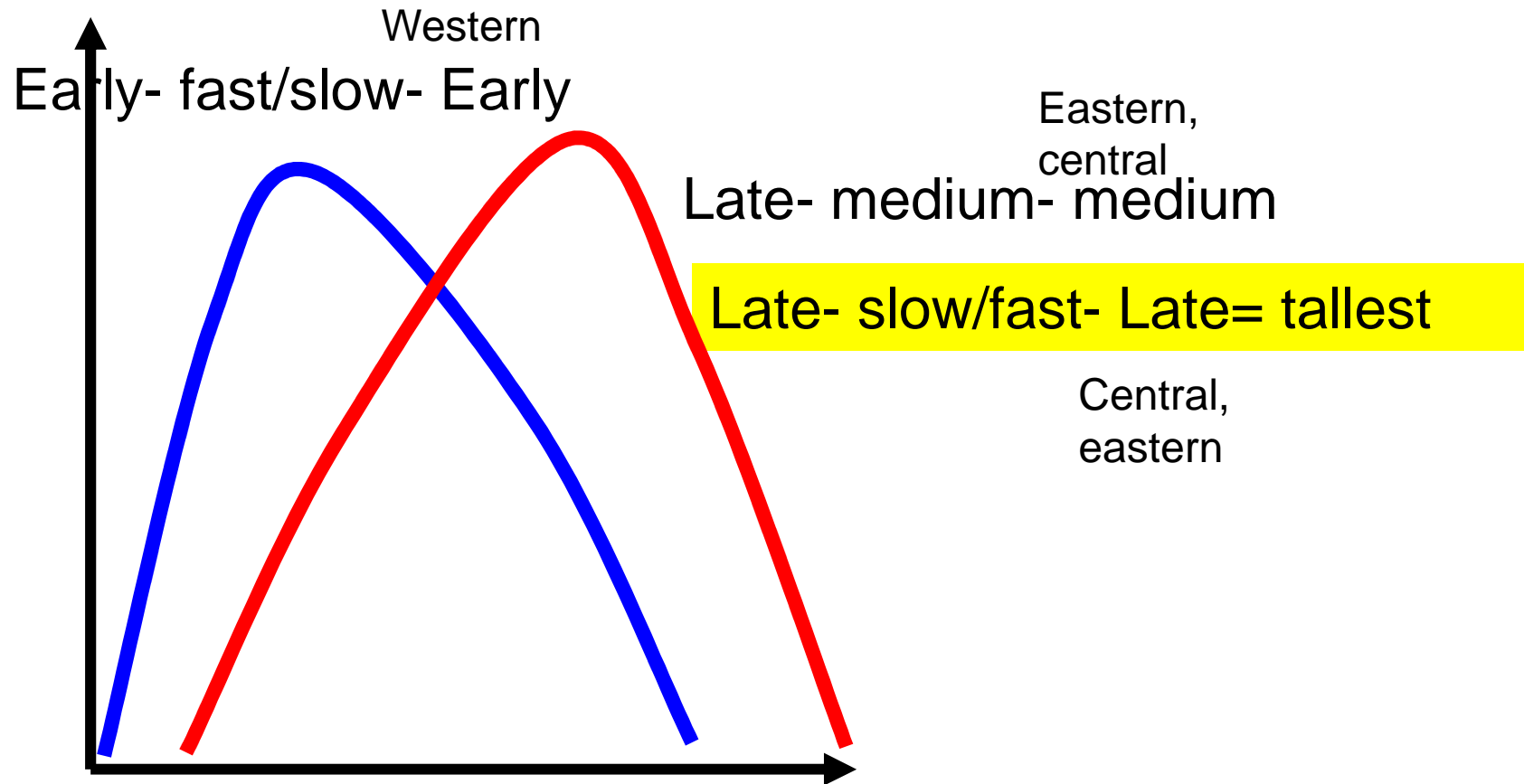




- Leader H = total H
- Effect of altitude after all?



Summary



Late start and end; together with fast growth at the second half of GP are the features of success (Ranki)

A photograph of a pine tree nursery. The trees are young and green, growing in a field of tall grass. A red circle highlights the top of a tree on the left side of the image. The background shows a dense forest of taller trees.

• Photoperiod is the same.

Why vary in growth cessation?

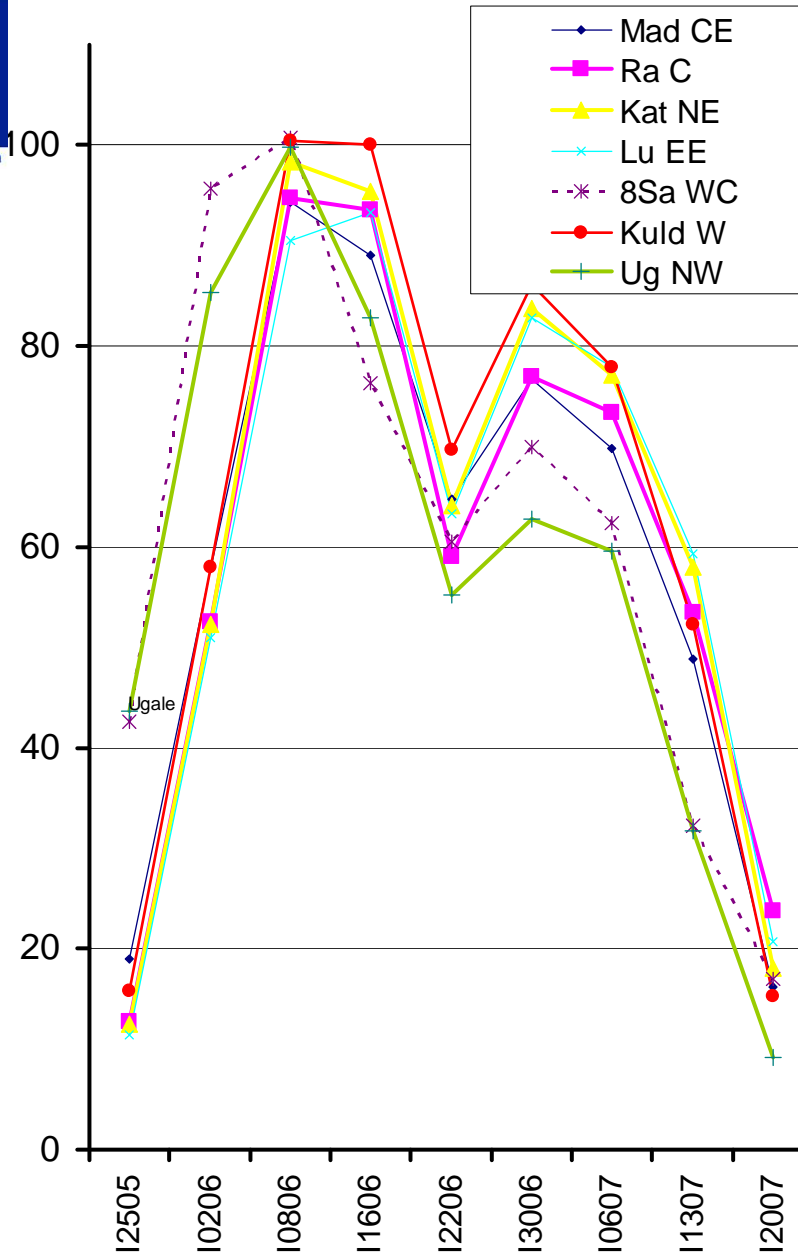
Free growth involved?

Bandomuosiuose želdiniuose



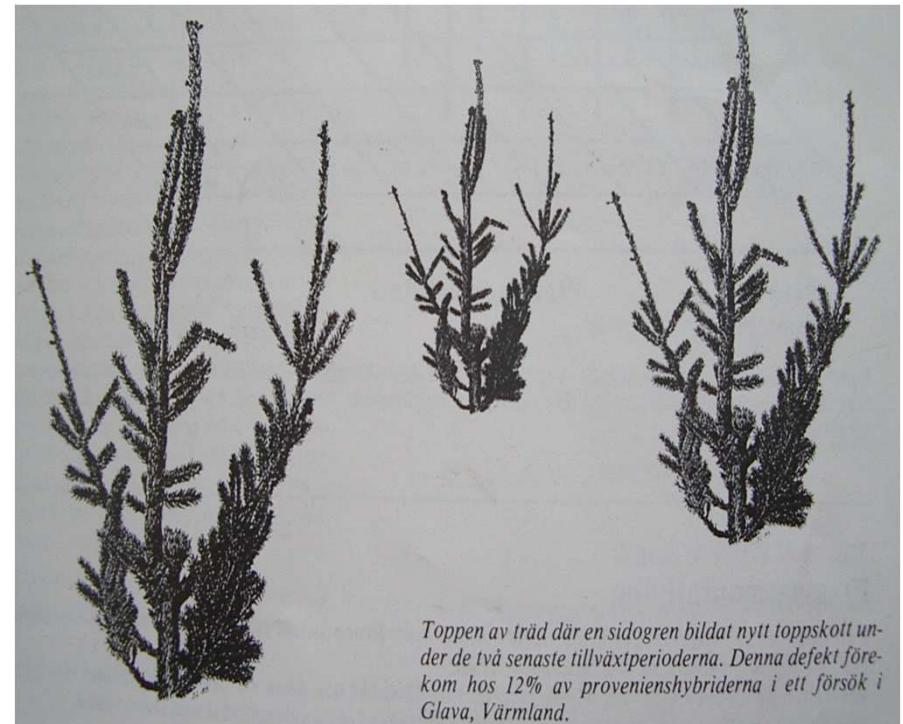
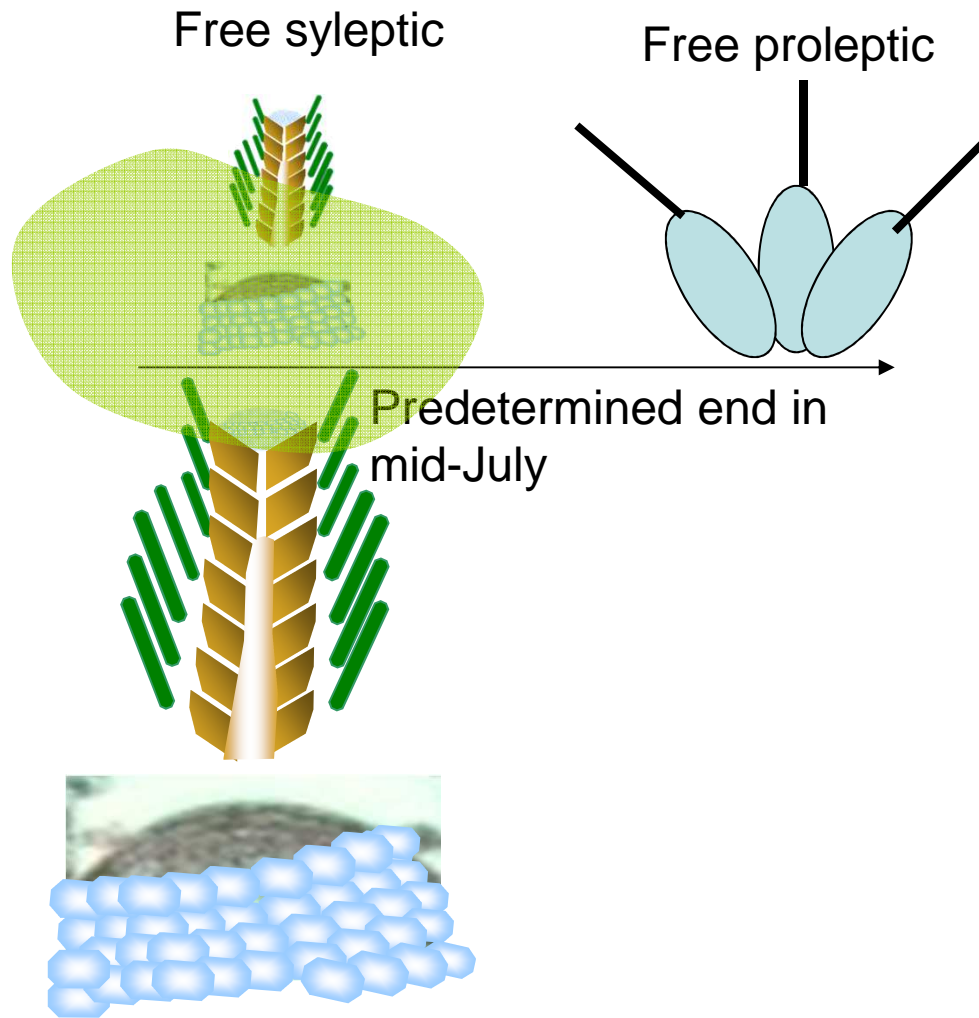
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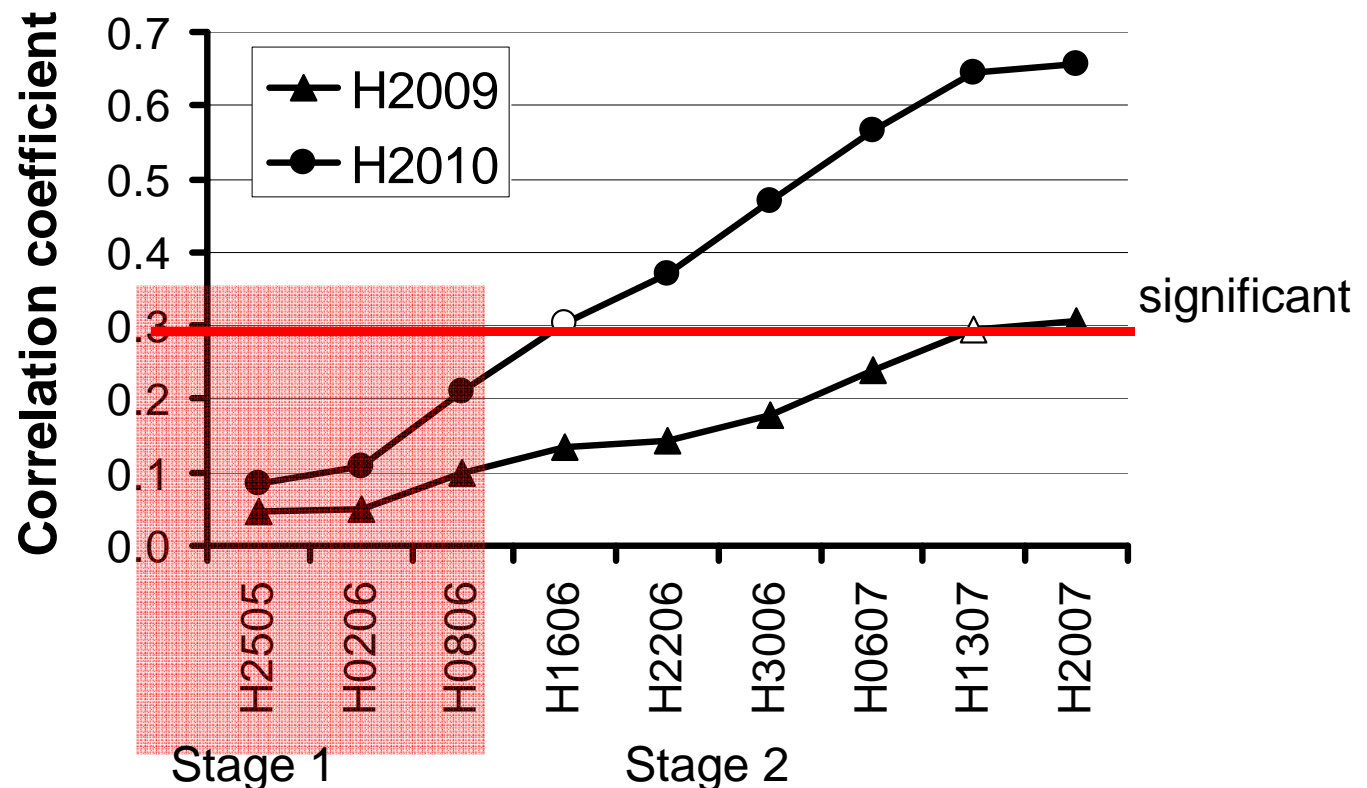
Early cessation = tendency for free growth

Shoot growth consists of predetermined and free (syleptic /proleptic) growth

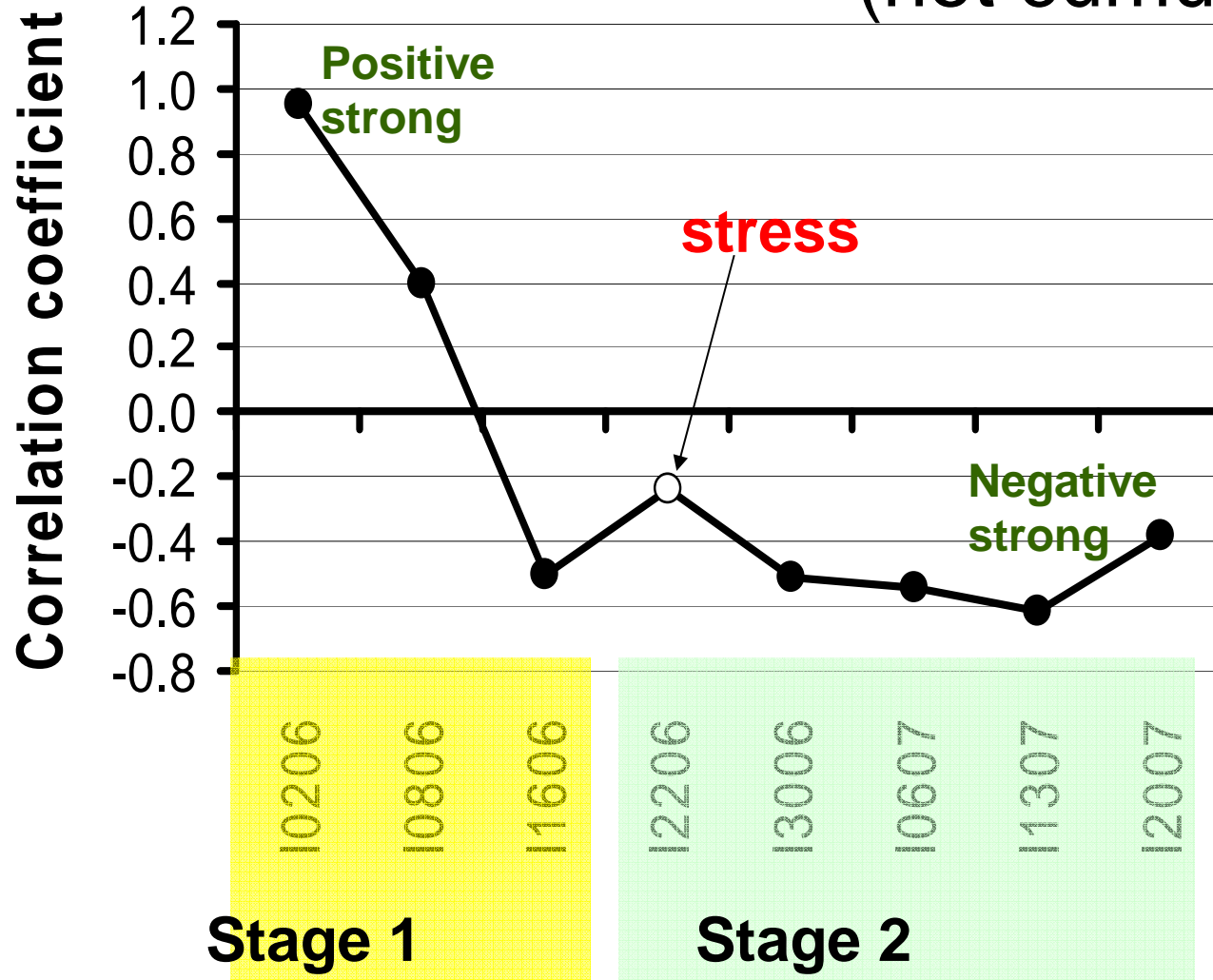


Correlations

- With what increments the Final H is correlated?
- Length of GP is more important than growth speed , because correlations tend to be significant at 2nd half of GP

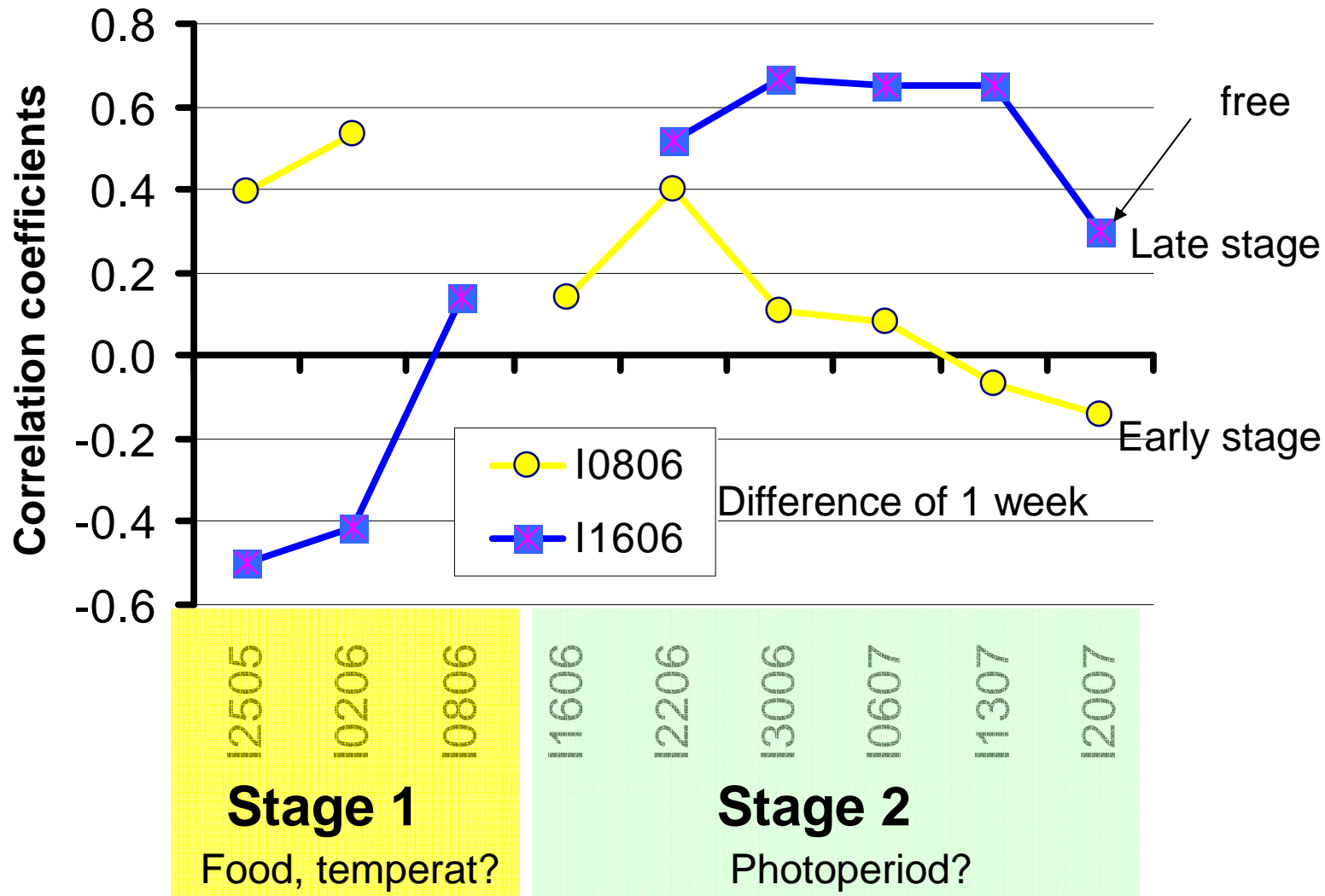


Corr between start and subsequent weekly increments (not cumulative)



- Early start means early cessation
- two uncorrelated growth stages:
 - 1 stage of fast initial elongation negatively correlated with later stages of elongation

Corrs among weekly increments given for two adjacent dates reflecting different growth stages





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Genetic parameters

Mixed model ANOVA

Cumulative increment				
Variable	Source	DF	F	P>F
H2009	REG	2	3.4	0.03403
H2010	REG	2	2.4	0.08721
H2009	POP(REG)	4	5.1	0.00043
H2010	POP(REG)	4	4.7	0.00091
H2009	FAM(POP)	53	2.9	0.00001
H2010	FAM(POP)	53	3.4	0.00001
H2009	REP	4	1.5	0.18614
H2010	REP	4	6.7	0.00002

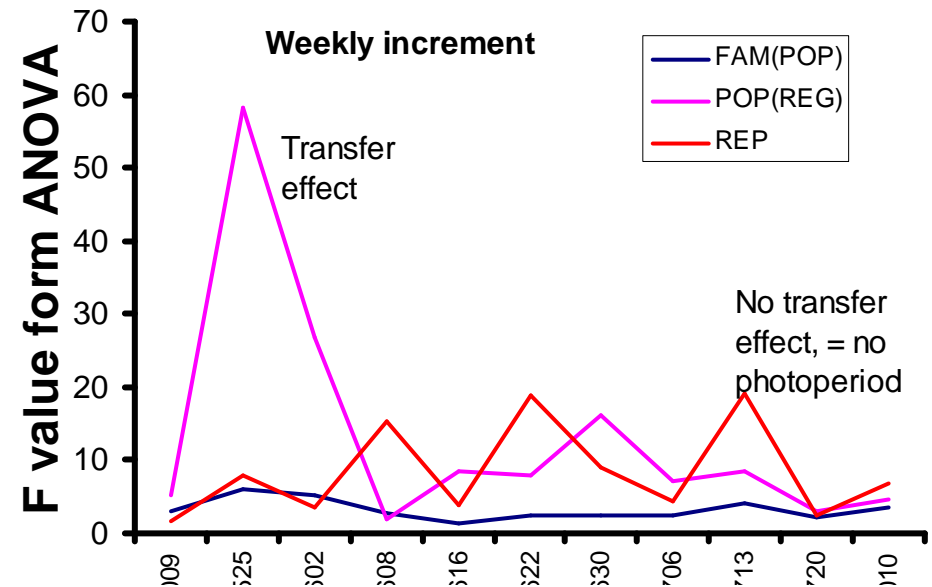
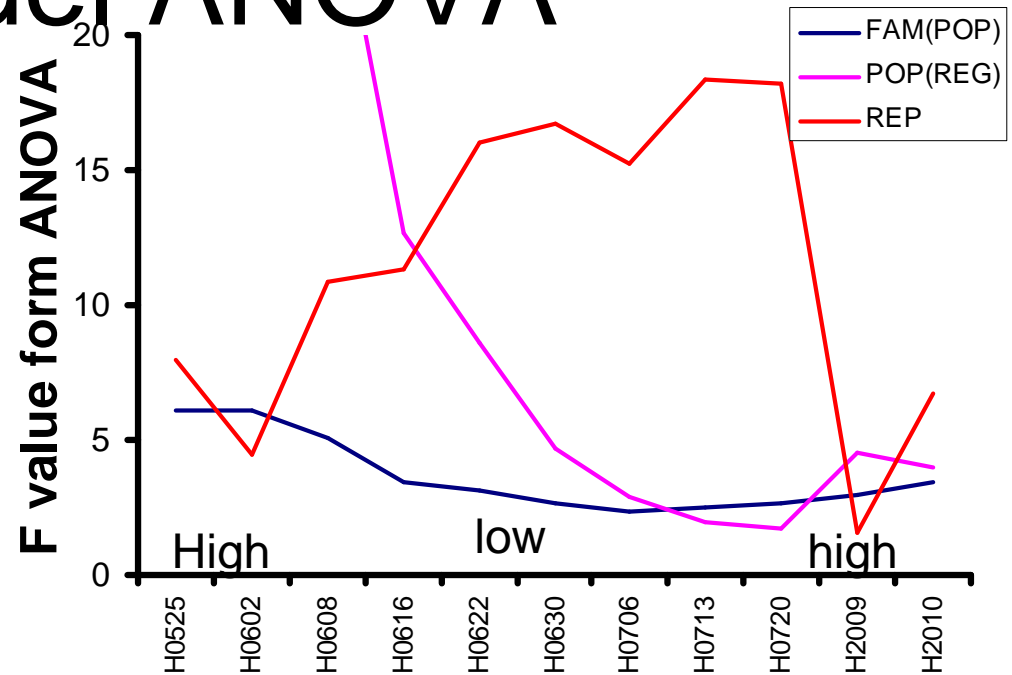
Tukey's Studentized Range (HSD) Test for variable: H0720

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than REG

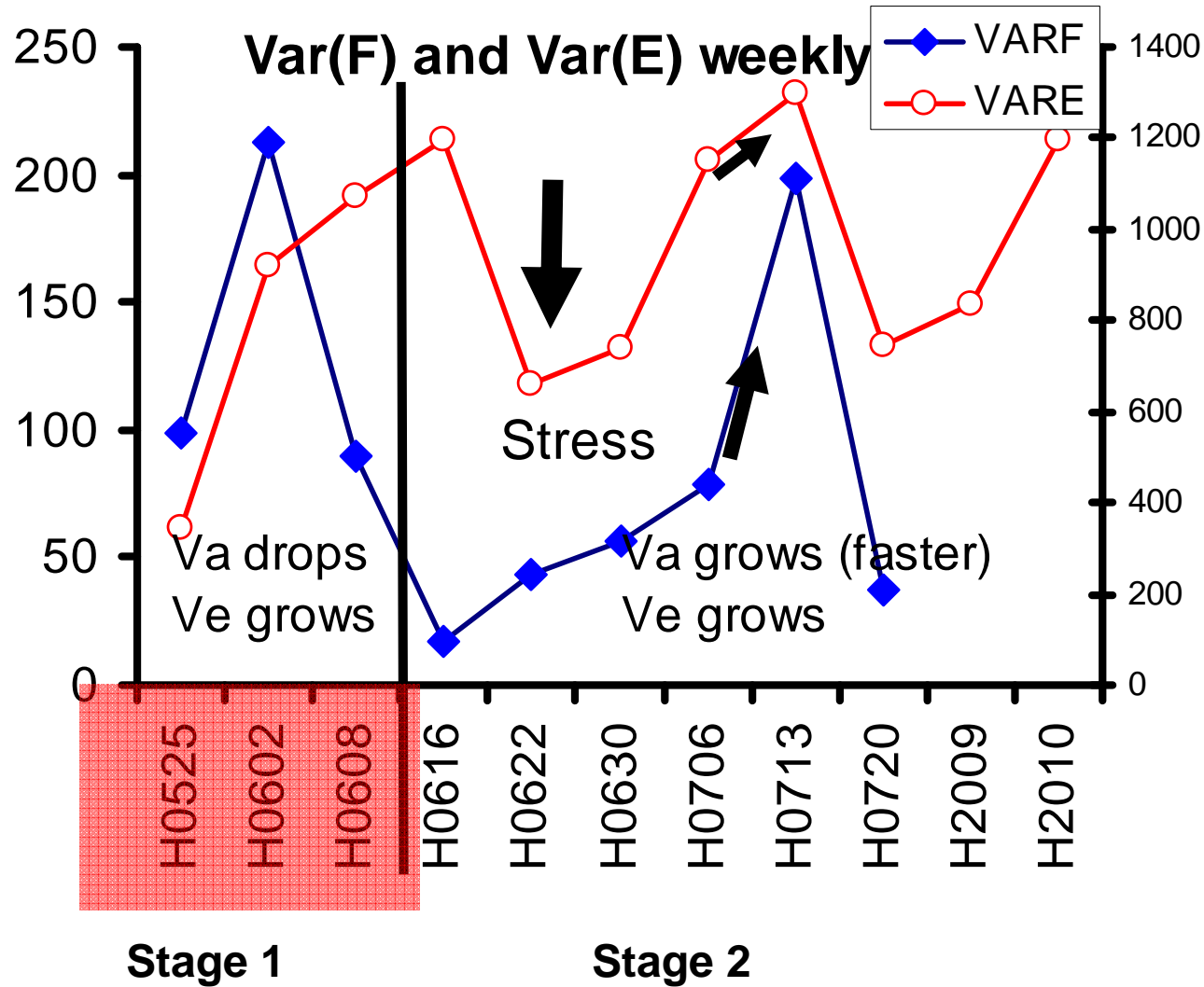
Alpha= 0.05 df= 1209 MSE= 27479.16
 Critical Value of Studentized Range= 3.864
 Minimum Significant Difference= 64.969
 WARNING: Cell sizes are not equal.
 Harmonic Mean of cell sizes= 97.17531

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	REP
A	587.17	342	2
A	582.52	346	1
B	548.53	295	3
B	499.73	26	5
C	480.28	264	4



Heritability variation

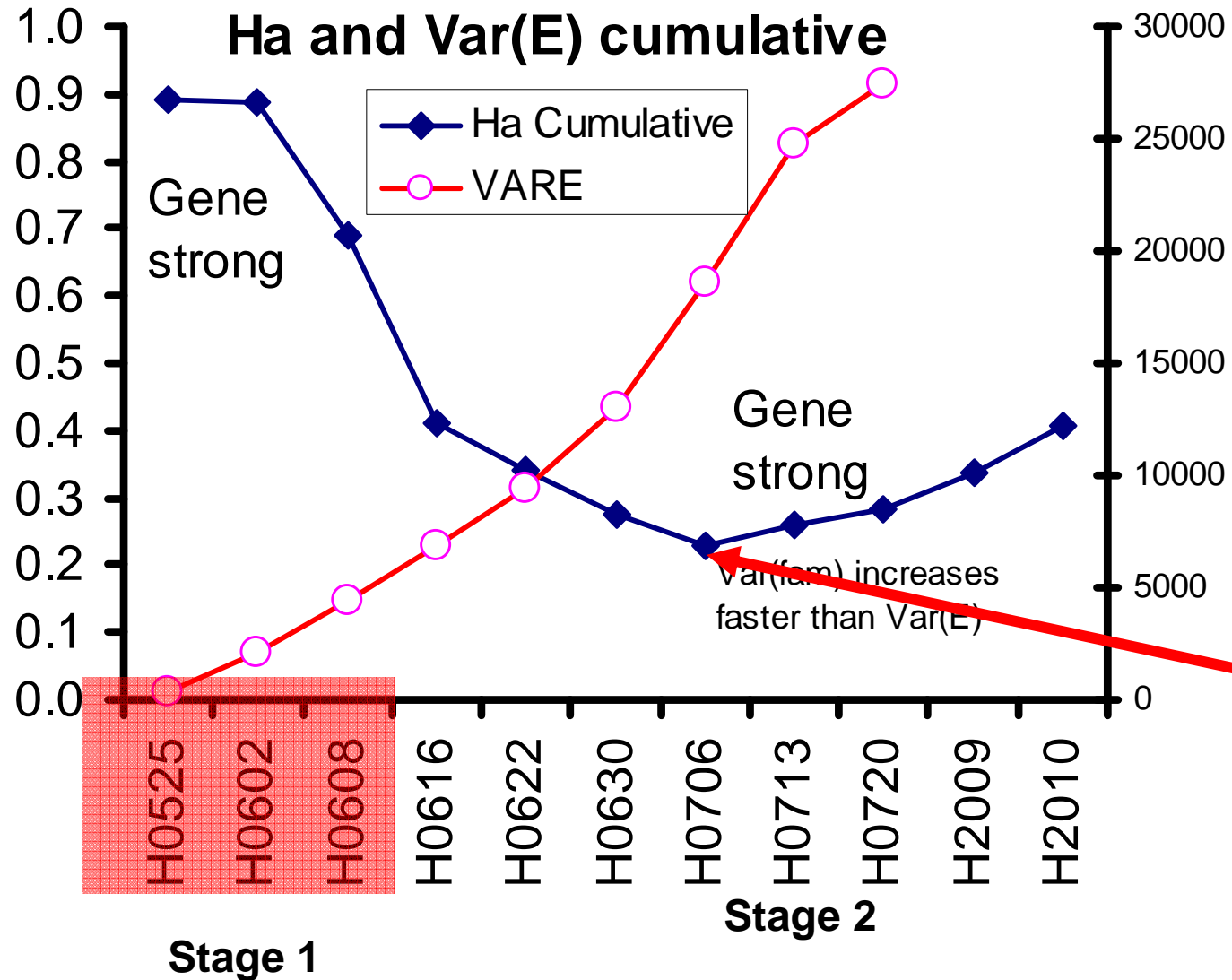


- Strong genetic control at start and end (= growth rhythm)

- Variances increase, but Va increases faster ?

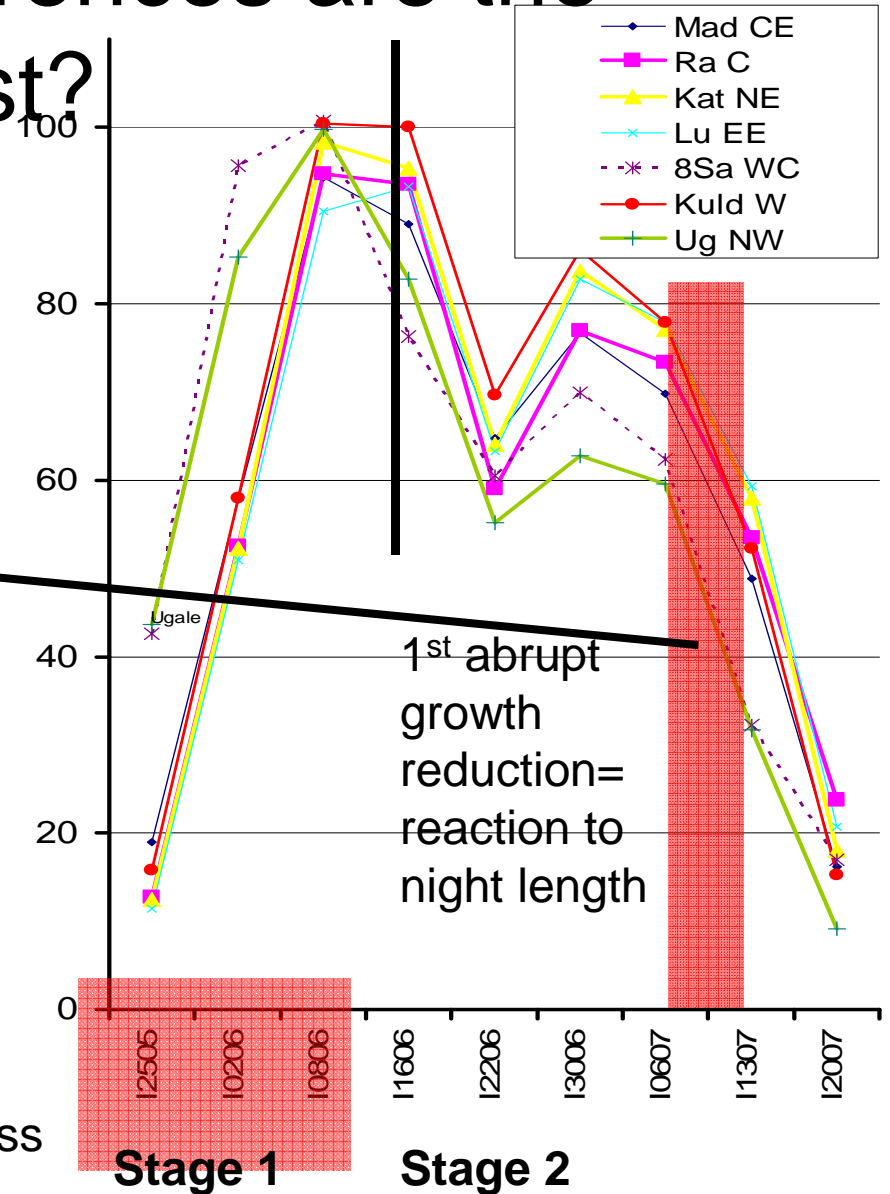
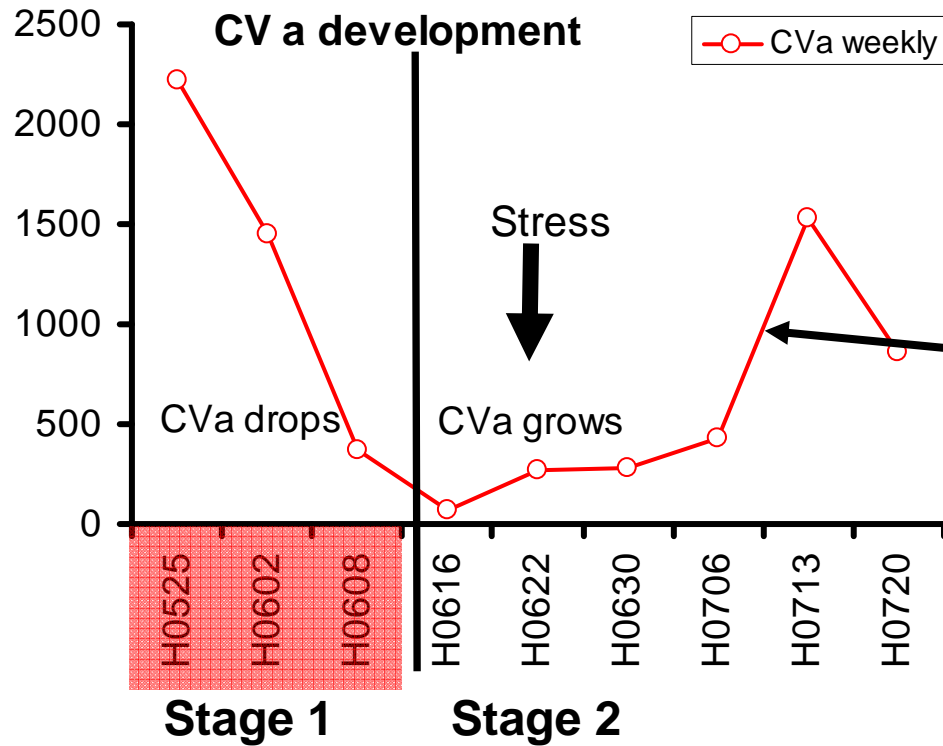
- Stress reduced Ve

Heritability variation (cumulative)



- Strong photoperiodic response manifested in 6th of July

When genetic differences are the greatest?



Gene differentiation increased at stage 2

Gene differentiation slight increased at stress

Gene differentiation sharp increase at 1st slow

Growth stages described

Stage1 Fast increment 06.16-22 Stage1 Slow increment

Night length

07.06 photoperiodic response

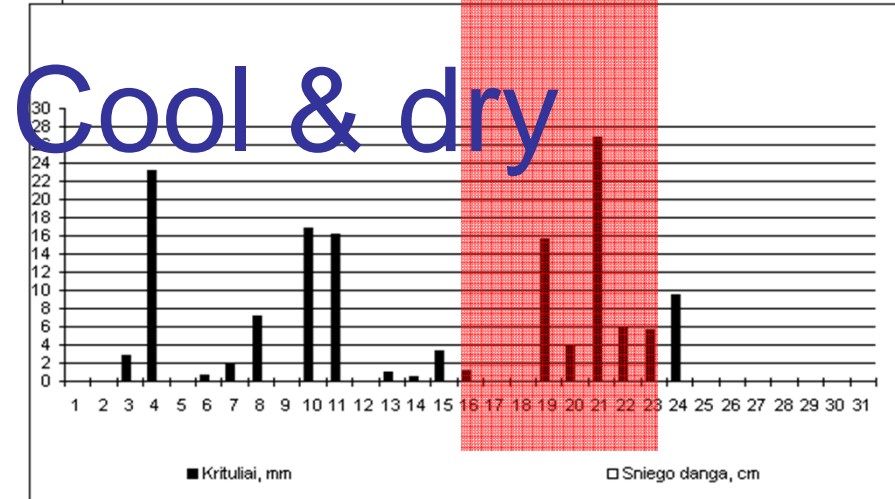
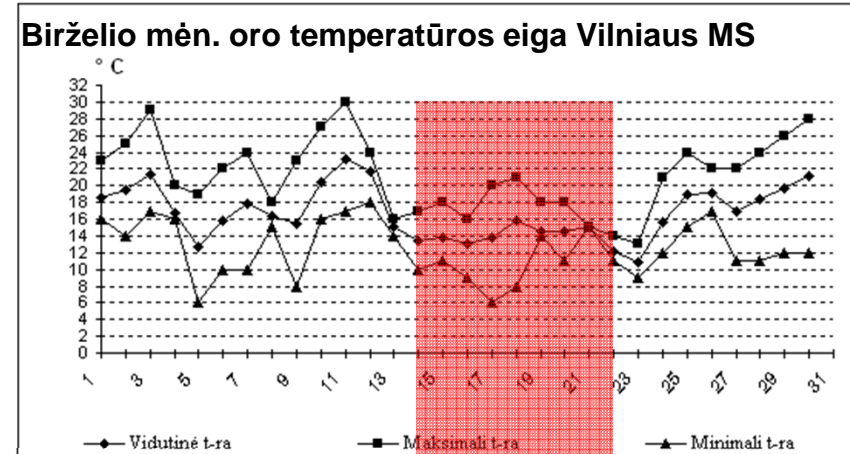
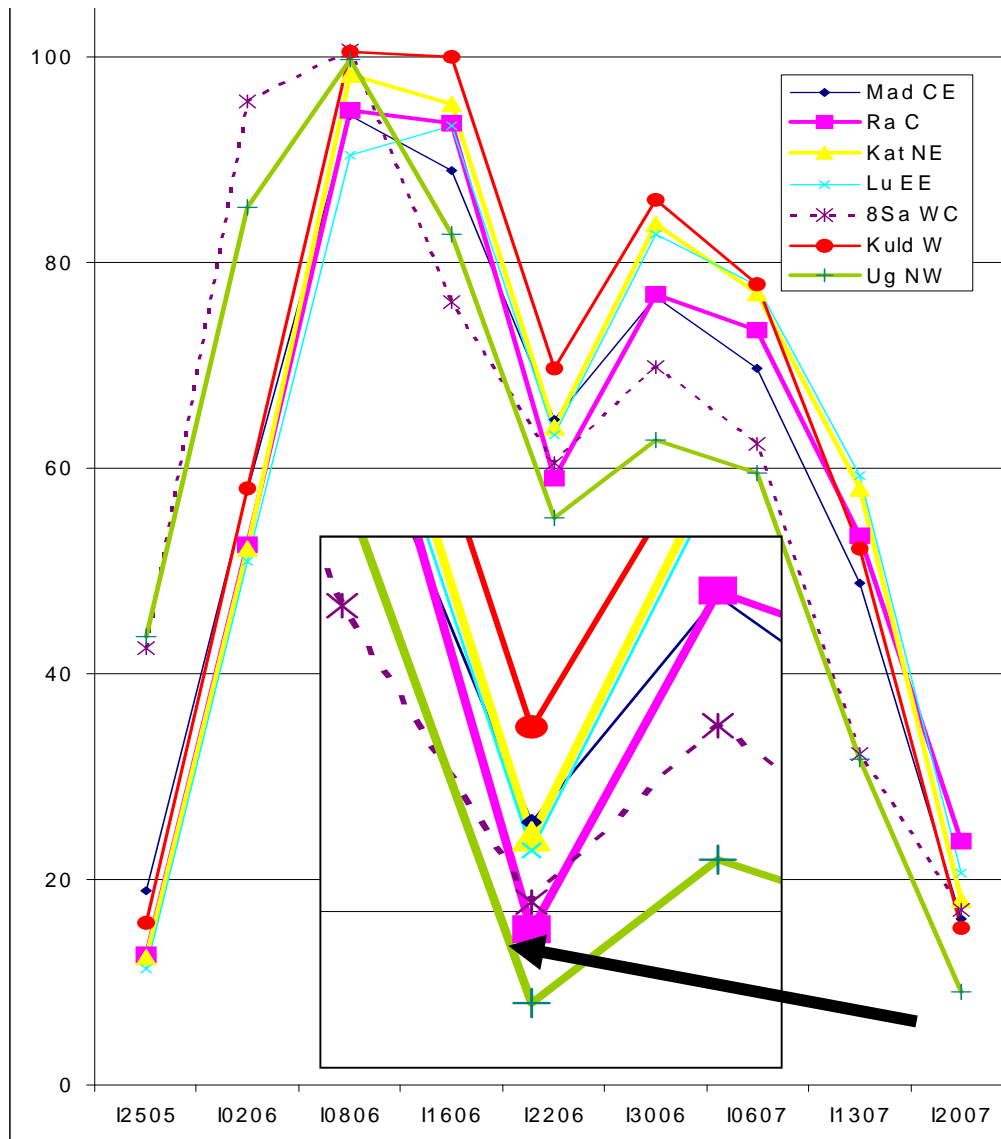


High ha
Genes

High ha
Genes

CVa drops	increases
Vf drops	increases
Ve increases	increases
Corr with start (+)	(-)

Stress 16-22 June










Worst for Ranki= the southern type

Conclusions





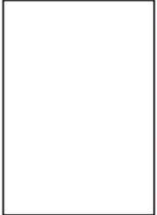



- Significant effect of altitude (may be 2 sub-zones)
- Population effect strong at start (tsum) but weak at cessation (photoperiod, free growth).
- Correlations: early start means early end.
- Ha and CVa lowest in the middle of GP.
- 2 growth stages:
 - fast growth, Ha, CVa drops, (-) corr with later stages
 - Slow growth: Ha, CVa increases (+) corr with later stages

The end

(vertinti viršūninį pumpurą ant viršūninio ūglio)

						
0 Niekio nevyksta	1 Pabrinke (balti, šv. rudi), spyglių nesimato	2 Pats sproginas: pumpuras sprogęs ir spyglių galai išlindę	3 Pirmas pailgėjimas: spyglių ilgis- 1-2 cm	4 Augimas: ilgis 3-4 cm, spygliai nėra taip sugludę kaip Nr. 3,	5 Ilgį ūgliai 5-7 cm : dėl pailgėjimo įgyja teptuko formą	6 Ūgliai >7 cm : yra naujo ūglio žalias stiebelis ir gali būti nauji pumpurai

Kokybė

							
0 Šalnų pakenkti ūgliai	1 Dvistiebis	2 2 ir > viršūninių ūglių	3 Liga, spygliai išblukę ar išdžiūvę	4 Viršutinė medelio dalis nudžiūvusi, auga iš apačios	5 Mechaniniai, žvėrių, kenkėjų pakenkimai	6 Viršūninis pumpuras pakenktas (vertintas šoninis pumpuras ant viršūninio ūglio)	7 Laisvas augimas ant viršūninio ūglio

Study carried out in European Social Fund's Project "Importance of Genetic Factors in Formation of Forest Stands with High Adaptability and Qualitative Wood Properties" (No 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146)



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Genome plasticity of Scots pine (*Pinus sylvestris* L.) under different stress conditions

Mag.Biol. Angelika Voronova

Dr.Biol. Dainis Ruņģis (LSFRI Silava, Genetic Resource Centre);

Dr.Silv. Āris Jansons (LSFRI Silava, Forest tree breeding)



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Introduction



Aegilops (wheat wild ancestor), <http://www.arcad-project.org>



- Short generation time
- Low genetic diversity
- Human selection since 8500 B.C. using phenotypic traits
- High specificity to environment
- Molecular markers for selection are broadly used



- Long generation time & Molecular markers
- High genetic diversity
- Natural selection
- high plasticity to environment
- Phenotypic traits selection should be used in association with molecular markers



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Retrotransposon variation



- mobile genetic elements
- replicative transposition
- the largest compound of plant genomes (15-90 %)
- cluster formation

? rearrangement of genome (instability), - functional mutations, - somaclonal variation, - recombination process, - genome structure upkeep

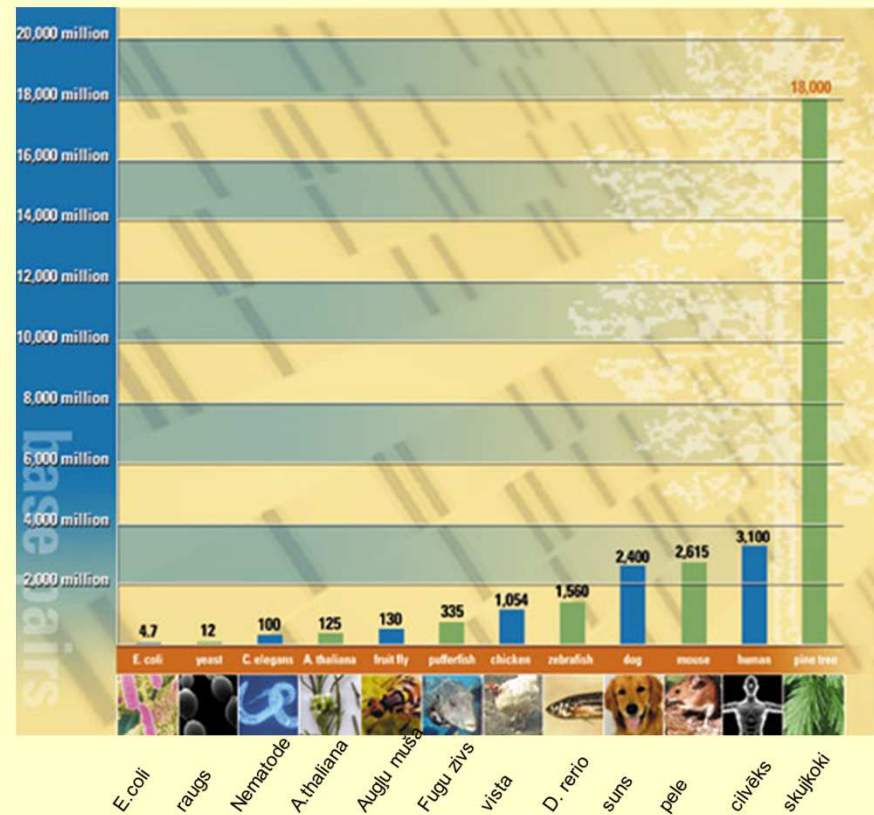
- transpositional activation observed in stress conditions

(McClintoc, 1984, Peschke et al. 1987, Grandbastien et al. 1998, Hirochika et al. 1993, Poteau et al. 1994, Ramallo et al., 2008).

Adaptive selection to larger genomes with higher retrotransposon activity?

Scots pine genome

- one of the ancient's plant groups gymnosperms (*Pinophyta*)
- $2n=24$ genome size:
 - 50 pg (Grotkopp et al, 2004);
 - 42,5 (Bogunic et al., 2003);
 - 55,6 (Valkonen et al. 1994).
- 70-75 % repetitive sequences
- *Pinus nigra* population study of variation in genome size (Bogunic et al. 2007) 0,64 pg 2,6%.
- variation is determined by spread of different sequence repeats as satellites and retrotransposons.





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Aims of study

Identify active retrotransposons in Scots pine (*Pinus sylvestris* L.) genome and characterize its structure, distribution and transcriptional rate during different stressors in controlled conditions.



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Brief structure of study



- ✓ Control molecular markers design
- ✓ Optimisation of methods;
- ✓ High temperature effects on the transcription of pine mobile genetic elements
 - Confirmation of clonal identity of the ramets;
 - Induction of stress conditions and RNA extraction;
 - Identification of mobile genetic elements;
 - Specific marker design;
 - Data collection and analysis.
- ▶ Biotic stress effects on transcription of pine mobile genetic elements;
- ▶ effects of Salicylic acid and ABA.



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Brief structure of study



- ▶ analyses of established molecular markers on the natural pine stands growing in different conditions;
- ▶ Full-size retrotransposon isolation and sequencing;
- ▶ ▶ Identification of retrotransposons/ classification/ structural studies / prevalence studies in the pine genome



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Scots pine ramets and stress initiation



Pine Woolly Aphid
(*Pineus pini*)



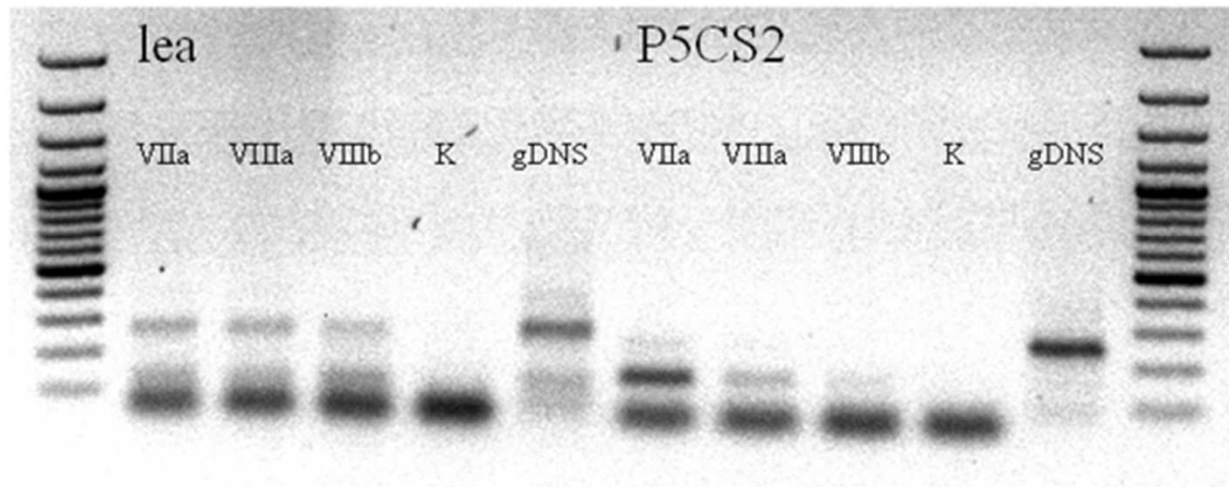
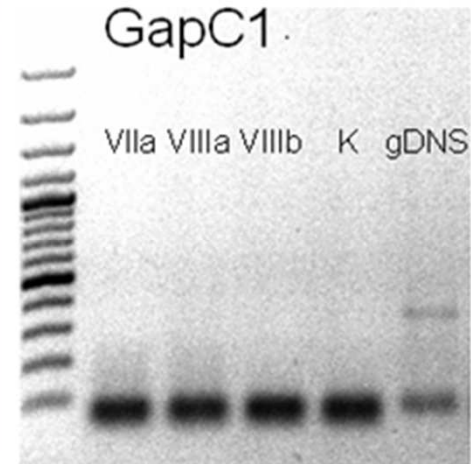
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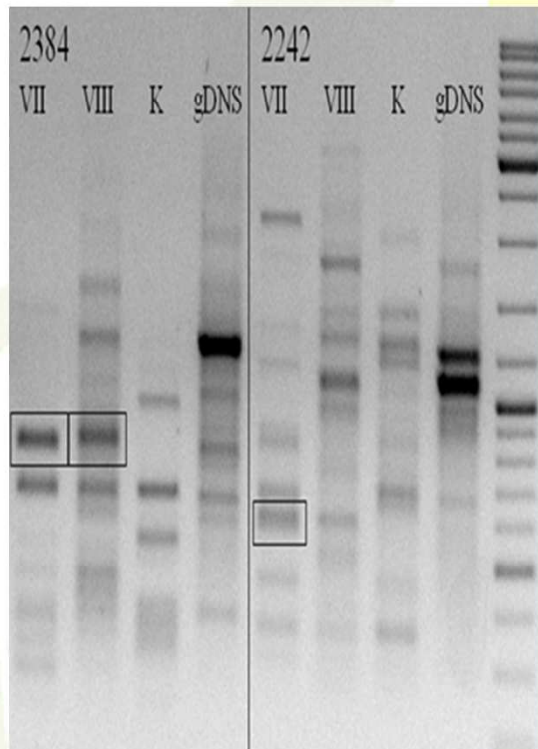
Control molecular markers design



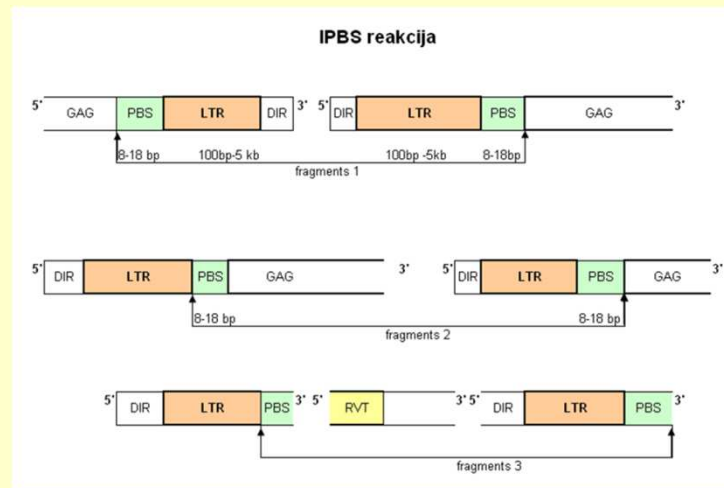
- dhn3 (AJ512362.1)
- abaH (FJ201653.1)
- Lea (FJ201577.1)
- P5CS2 (NM_115419.4, EF412967.1)
- GapC1 (L07501.1 Jaakola et al. 2004)
- pns (S50350.1)
- lp3 (U67135.1)



Methods



iPBS reaction (Kalendar et.al., 2010);



Inter PBS amplification. Lanes VII and VIII show fragments amplified from cDNA samples from heat stressed trees, lane K is the cDNA sample from the control tree, lane gDNS shows amplification with genomic DNA of the same ramet, and the last lane is size marker GeneRuler DNA Ladder Mix (Fermentas). Excised fragments are indicated.

Classification of mobile genetic elements

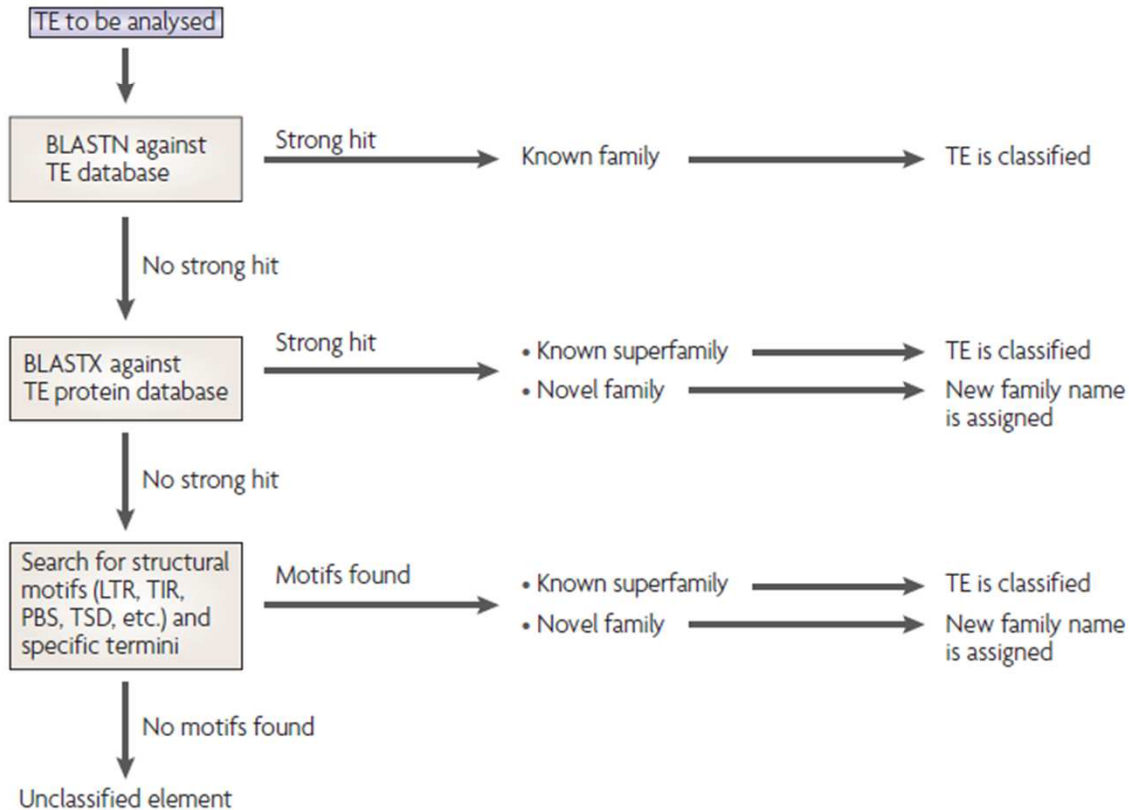


Figure 3 | Step by step transposable element (TE) classification. LTR, long terminal repeat; PBS, primer binding site; TIR, terminal inverted repeat; TSD, target site duplication.

Wicker et al., 2007



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Sequence analyses

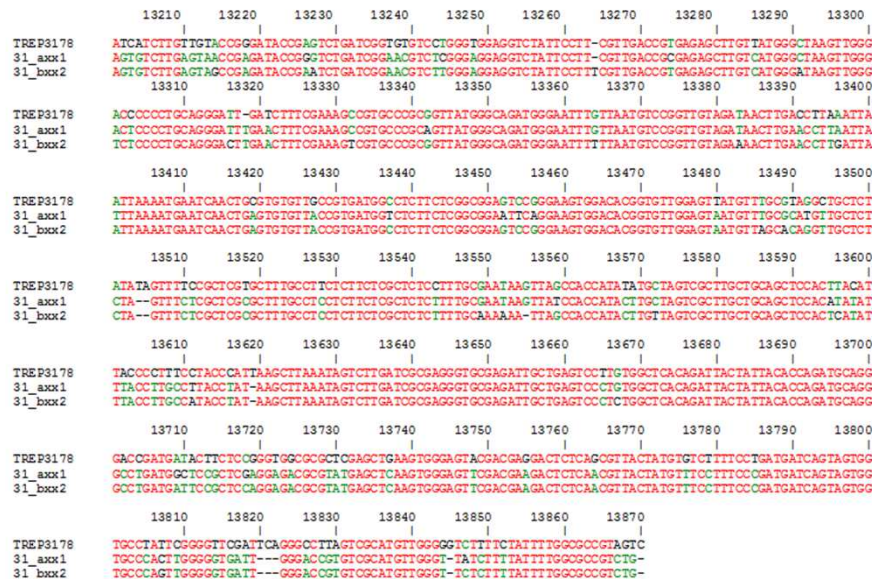
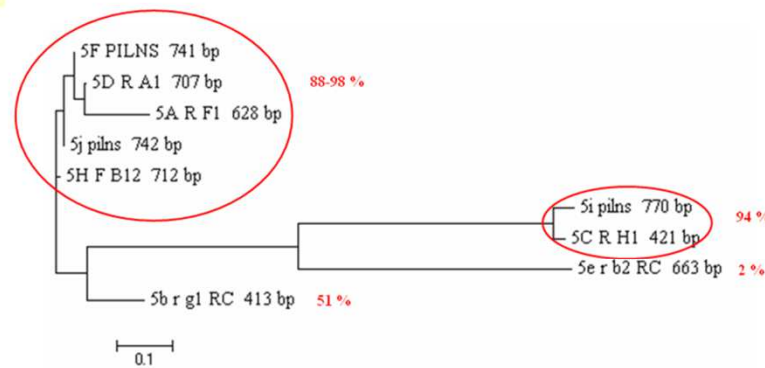


Figure 3. Multiple sequence alignment of 3.1 group fragments with LTR Gypsy, Erika TE (data base number: TREP3178) nucleotide sequences.



>nl|PTREP|PTREP173 DNA transposon, TIR, CACTA, "DTC_Conan_consensus-1";
 (TREP3415) ORF2 protein Length = 1124
 Score = 274 bits (701), Expect (2) = 2e-89
 Identities = 170/228 (74%), Positives = 188/228 (82%), Gaps = 2/228 (0%)
 Frame = +2

Query: 101 PRCRTWYFAYHGGG-LDQKTGKISWKASLKGAKQKIIDTIEEERRGFMPNRENDELTCAL 277
 PRCRTWYFAYHGG LD KTG +S KA L GA ++ IEE+R G F PNRENDEL AL
 Sbjct: 277 PRCRTWYHGGGELDPKTNVSTKACLNGADDALLVAIEEARSQVFPNRENDELTRAL 336

Query: 278 GNPEYQGRTRGKGVIPWYEGFSDMNDYRARKKMEEEKRKLLEEQRKQDAERLQGLE 457
 GNPE+ GRTRGK IPWYEGFSDMN DYR+RARKK+ EEKRRK+EEEQRK D ERLQGLE
 Sbjct: 337 GNPEHPGRTGKGAIPWYEGFSDMNADYRTRARKKIAEKKRKLLEEQRKLDYERLQGLE 396

Query: 458 ARHADLALFKFRQQQ*IDSLSEQRSQQRQQQADDRPALDSTVPSMRRSSVGSAPGD-TL 634
 A A+LA KF++QQ+ IDSLSQ+RGSQQ QQ ADD PALD+T PSNRRSSVGSAPGD +
 Sbjct: 397 ASQAEELAAKFORQQEQIDSLSQRGSQQQLQLADD-PALD+TAPSRRSSVGSAPGDVAV 455

Query: 635 LDTPVDDIIEDTNCELHSHKMNISHKQVADGVAFPVTPRATYHCIP IP 778
 LD YPVDDI E+TNCELH KHKNISHKQVAD VAF +P AT+HC PIP
 Sbjct: 456 LDRYPVDDITENTNCELHFHKMNISHKQVADAVAFNSPEATFHCNPIP 503

Searches were done in the

- NCBI data base (<http://www.ncbi.nlm.nih.gov/BLAST/>);
- GrainGenes Triticeae Repeat Sequence Database (<http://wheat.pw.usda.gov/ITMI/Repeats/blastrepeats3.html>);
- Gypsy Database 2.0 (<http://gydb.org/index.php/Blast>) (Llorens et al., 2011).



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Results of similarity searches



Fragment	LTR elements		Non-LTR elements	DNA transposable elements	Unclassified elements	EST	No similarity	Blastn: Score/E-value/Positives	Blastx: Score/E-value/Identities/Positives
	Gypsy superfamily	Copia superfamily	LINE	CACTA					
1.1							•	-	-
1.2		•						-	135/3e-34/41%/61%
2.1	•				•	•		-	55.1/1e-09/29%/43%
2.2						•		-	-
3.1	•							670/0.0/88%	-
3.2	•				•	•		-	54.7/2e-09/29%/43%
3.3	•							-	53.5/3e-09/28%/47%
4				•				620/e-178/95%	274/2e-89/74%/82%
5.1			•					56/6e-08/86%	179/3e-47/67%/77%
5.2							•	-	-
5.3							•	-	-
6.1							•	-	-
6.2						•		-	-
7.1						•		-	-
7.2	•					•		-	-
7.3	•							722/0.0/93%	-
8	•				•	•		-	52.8/6e-09/31%/43%
9.1				•				680/0.0/96%	295/4e-82/68%/76%
9.2	•					•		50.1/1e-06/83%	89/1e-20/52%/64%
9.3		•						-	-
10.1	•					•		686/0.0/90%	-
10.2		•						-	84.7/8e-19/59%/73%

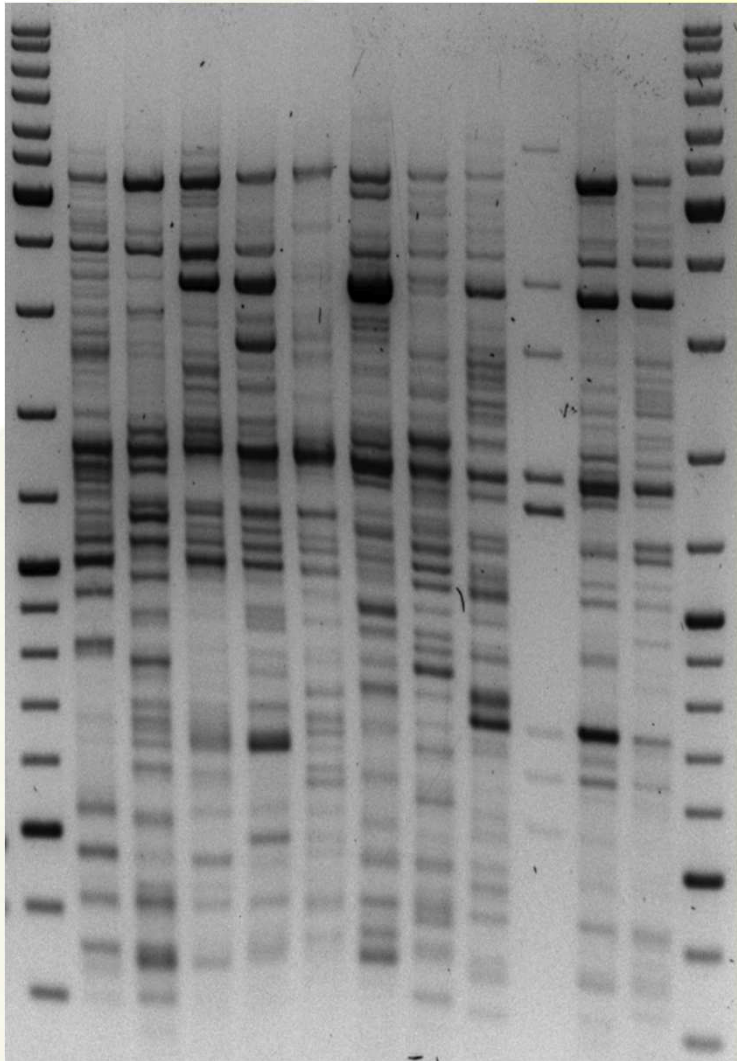


Classification of analyzed fragment sequences according to transposable elements from the TREP and GyDB databases



Fragment	Order, Superfamily	Name of TE	Domain	Data base	Organism
1.2	LTR, copia	<i>HORPLA2</i>	polyprotein	TREP, blastx	<i>Hordeum vulgare</i>
	LTR, copia	<i>BARE1</i>	polyprotein	TREP, blastx	<i>Hordeum vulgare</i>
	LTR, copia	<i>Orycol-1</i>	INT	GyDB, cores	<i>Oryza sativa</i>
	LTR, copia	<i>Tnt-1</i>	INT	GyDB, cores	<i>Nicotiana tabacum</i>
2.1/ 8/ 3.2	LTR, Gypsy	<i>Geneva</i>	GAG	TREP, blastx	<i>Hordeum vulgare</i>
	LTR, Gypsy	<i>Sabrina</i>	polyprotein	TREP, blastx	<i>Triticum turgidum</i>
3.1	LTR, Gypsy	<i>Erika</i>	genomic	TREP, blastn	<i>Triticeae</i>
3.3	LTR, Gypsy	<i>Sabrina</i>	polyprotein	TREP, blastx	<i>Triticum turgidum</i>
	LTR, Gypsy	<i>Diaspora</i>	GAG	GyDB, cores	<i>Glycine max</i>
4/ 9.1	DNS, TIR, CACTA	<i>Conan</i>	pol	TREP, blastn	<i>Triticeae</i>
5.1	LINE	<i>Persephone</i>	genomic	TREP, blastn	<i>Hordeum vulgare</i>
	LINE	<i>Karin</i>	polyprotein	TREP, blastx	<i>Hordeum vulgare</i>
7.3	LTR, Gypsy	<i>Wham</i>	genomic	TREP, blastn	<i>Triticeae</i>
9.2	LTR, Gypsy	<i>Ifis</i>	genomic	TREP, blastn	<i>Triticum turgidum</i>
	LTR, Gypsy	<i>Carmilla</i>	polyprotein	TREP, blastx	<i>Triticum aestivum</i>
10.1	LTR, Gypsy	<i>Laura</i>	genomic	TREP, blastn	<i>Triticeae</i>
10.2	LTR, copia	<i>Maximus</i>	polyprotein	TREP, blastx	<i>Triticum aestivum</i>

Inter Retratransposon amplification



Nine newly developed Retrotransposon Markers

184 fragments were analysed

150 samples from one natural pine stand growing in highland, slope and lowland.

355-279 more fragments were found in the samples from trees growing in highland

Stands	heat stress markers		biotic stress markers		Total
	Sum 40	Average	Sum 38	Average	
pop_highland	2269	56,73	2281	60,03	4550
pop_slope	2173	54,56	2098	55,29	4271
pop_lowland	2135	53,59	2060	54,30	4195



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Conclusions



- Unspecific iPBS amplification reveals significant retrotransposon variation in Scots pine genome;
- Representatives of different families of Retrotransposons are found. Three fragments could be classified to particular TE families with high probability as they showed high similarity at the nucleotide level (LTR, *Gypsy, Laura*; LTR, *Gypsy, Wham*; DNS, TIR CACTA, *Conan*; LTR, *Gypsy, Erica*).
- Analysed sequences are consistently transcribed under various stress conditions as analysed sequences shows similarity with EST database sequences derived from cDNA libraries obtained in various studies of stress responses.
- The presence of sequence variation indicate that the transcripts originate from different copies in the pine genome.



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Conclusions



- One of the analysed sequences showed a similarity to a DNA transposable element *Conan*. Due to the clustering of retrotransposons within genomes, the identified sequences could be fragments or inactive elements within an active element.
- Some of the analysed sequences were similar to several known active retroelement proteins (*BARE-1*, *Tnt-1*) which could indicate that these retroelements possess transposable activity.
- These results indicate that further isolation of complete elements is needed to prove their transcriptional activation and to investigate activation of these elements in differing stress conditions.
- Due to high genetic diversity to access differences in natural pine stands affected by retrotransposition more molecular marker needed or we need to choose more homogeneous sample set.



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Presentation of Results



- **Expression of retrotransposon-like sequences in Scots pine (*Pinus sylvestris*. L) in response to heat stress.** 2011. A.Voronova, Ā.Jansons, D.Ruņģis, Environmental and Experimental Biology, in press.
- 4.2.2010.- 68. Scientific Conference of University of Latvia, Plant biology section.
- 22.-24.04.2009.- 5th International Conference “Research and Conservation of Biological Diversity in Baltic Region”, Daugavpils, Latvia (Oral presentation).
- 19.-21.03.2009.– FEBS Workshop „Adaption Potential in Plants”, Vienna, Austria (Poster presentation).
- 4.02.2009.– 67. Scientific Conference of University of Latvia, Plant biology section (Oral presentation).



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Acknowledgements



- This study was supported by the European Social Fond project (No. 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146).



- I am thankful to Dr. silv. Imants Baumanis for providing the experimental pine ramets for this study and to Dr. biol. Nils Rostoks for assistance in transformation assay setup and team of Genetic Resorce Centre of LSFRI Silava





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Spruce. Natural durability

Ilze & Co

ESF 146 seminar 30.11.-01.12. 2011

Silava, Kalsnava



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“Natural durability: the inherent resistance of wood to attack by wood destroying organisms” (EN 350)

LVS EN 350-1:2000. Durability of wood and wood-based products - Natural durability of solid wood - Part 1: Guide to the principles of testing and classification of the natural durability of wood

LVS EN 350-2:2000. Durability of wood and wood-based products - Natural durability of solid wood - Part 2: Guide to natural durability and treatability of selected wood species of importance in Europe

LVS EN 113:2000. Wood preservatives - Test method for determining the protective effectiveness against wood destroying basidiomycetes - Determination of the toxic values

The **AIM**

to determine natural resistance of particular spruce clones regarding fungal degradation

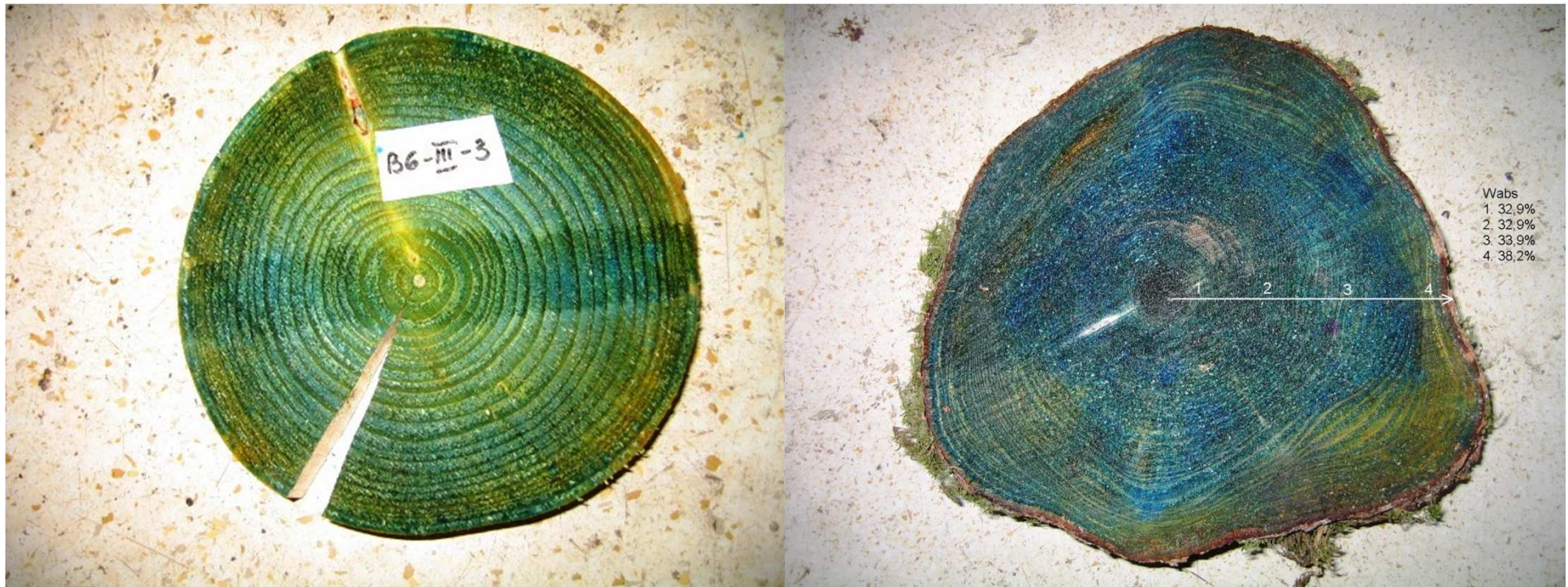


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Sapwood/ heartwood

Bromcresol green: heartwood – blue; sapwood - yellow
(KUTSCHA and SACHS, 1962).



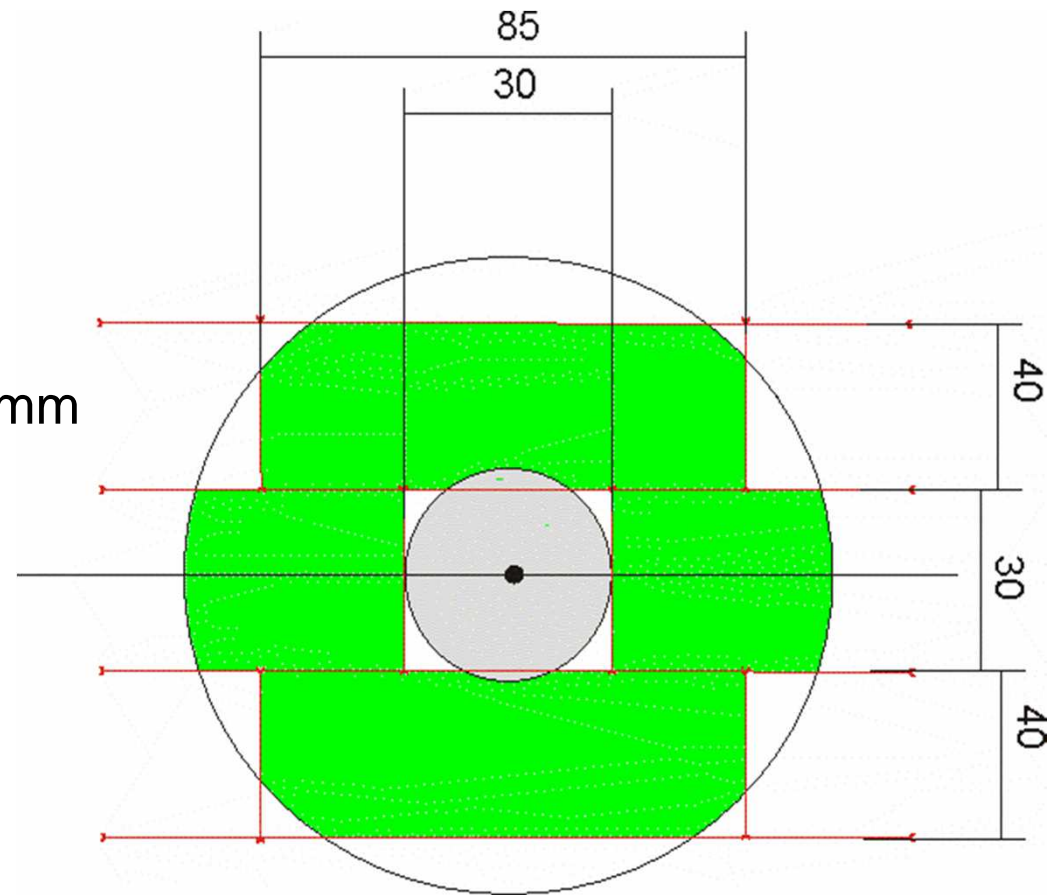
ESF spruce ~ 25 years

Spruce > 60 years

Natural durability tests:

- 10 Norway spruce (*Picea abies*) clones: 26, 31, A10, A15, A7, B10, B15, B6, V7, V9
- Control – Scots pine (*P. sylvestris*) sapwood

Tree division in laths, mm





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Drying-kiln, at 60°C



Specimens at 12% moisture (m/m): 50 x 25 x 15 mm
Theoretical volume: 18,75 cm³

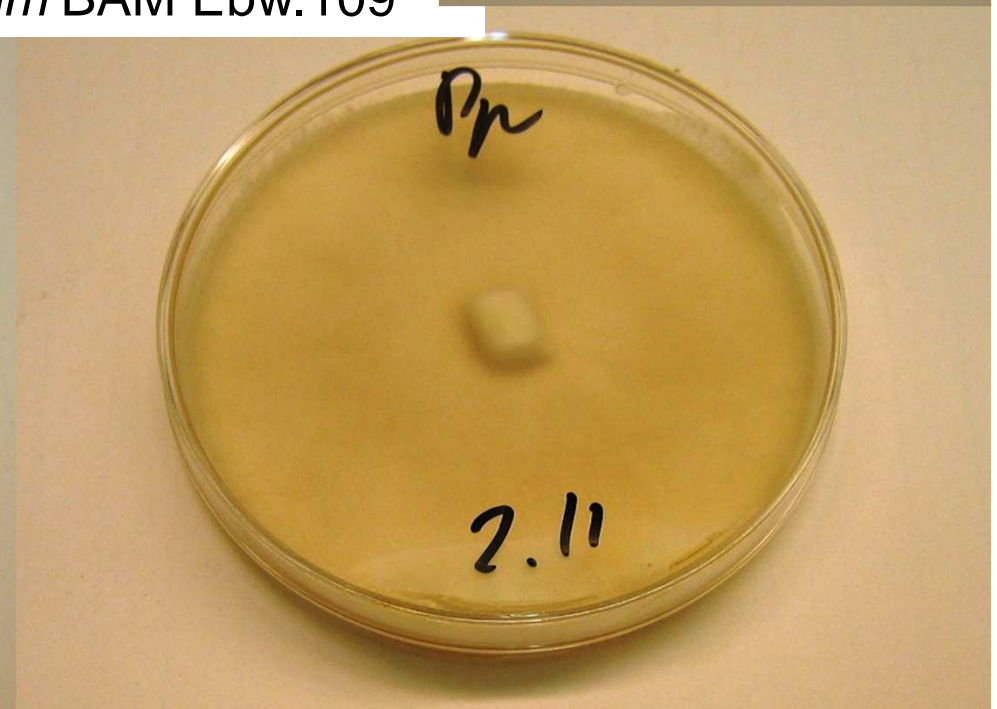
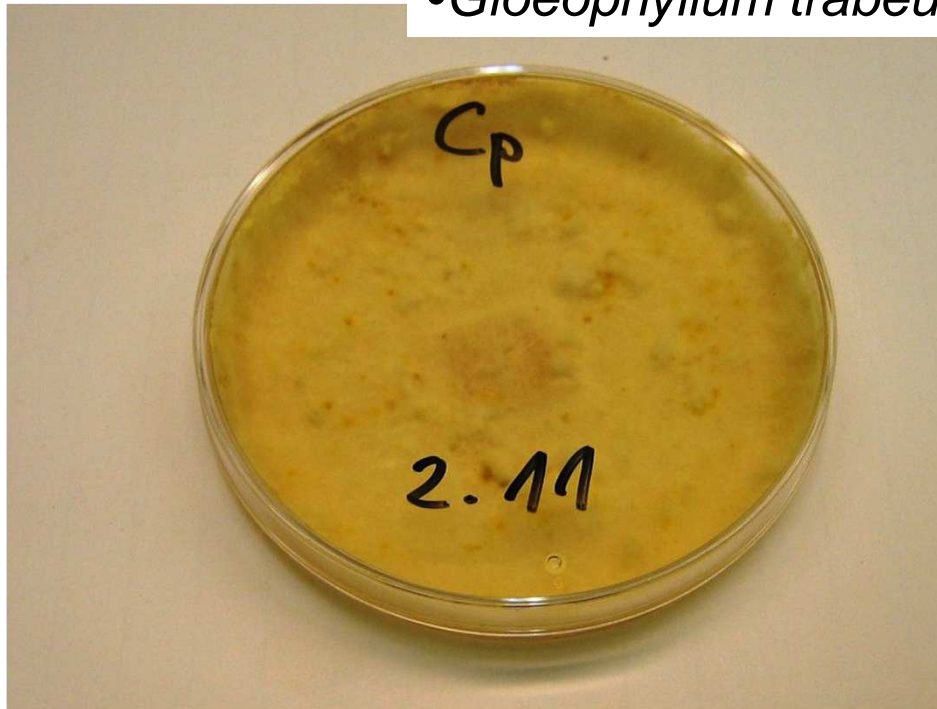


Wood quality:

- Free from cracks, stain, decay, insect damage or other defects
- Growth rings in any direction with exception of completely tangential orientation on the broad faces
- Longitudinal faces parallel to the direction of grain

Fungal cultures for conifers:

- *Coniophora puteana* BAM Ebw.15
- *Poria placenta* FPRL 280
- *Gloeophyllum trabeum* BAM Ebw.109





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Kolle flasks



Malt-agar medium

Fungal culture

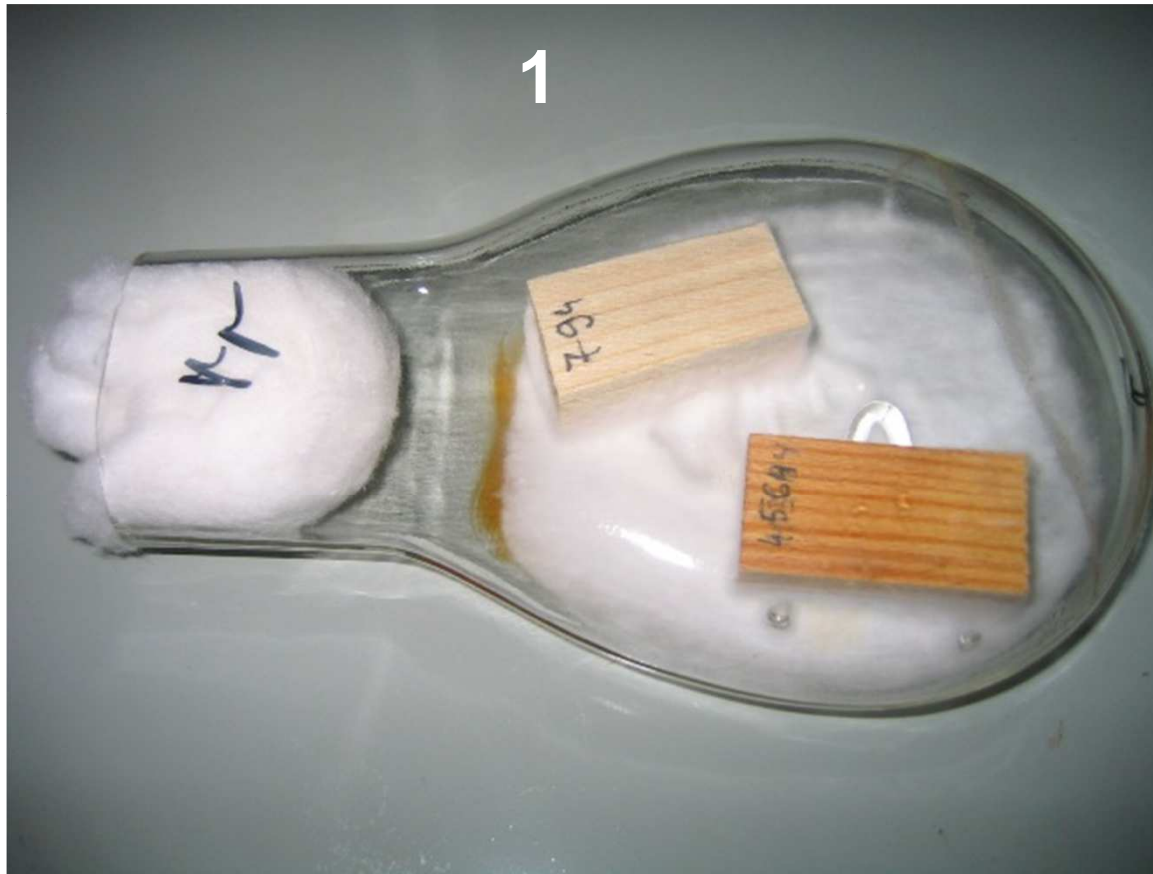




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Test wood (spruce) + control (pine)





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Test conditions: 22°C/ 70%RH
Duration:16 weeks



Coniophora puteana



Poria placenta



Gloeophyllum trabeum

Criterion – wood biodegradation i.e. mass loss after the test



Spruce before test

*After test (*P. placenta*)*



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EN 350-1. Classification of the natural durability to wood-destroying fungi

Durability class	Description	Lab test results given as X-value*
1	very durable	$X \leq 0.15$
2	durable	$X > 0.15$ but ≤ 0.30
3	moderately durable	$X > 0.30$ but ≤ 0.60
4	slightly durable	$X > 0.60$ but ≤ 0.90
5	not durable	$X > 0.90$

*X = mean mass loss of test wood/ mean mass loss of control



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Summary results Spruce durability vs density, extractives, lignin

Spruce clones*	<i>C. puteana</i> x – value	<i>P. placenta</i> x – value	<i>G. trabeum</i> x-value	Density kg/m ³	Extractives %	Lignin %
A 7	0.97	1.06	0.97	405.8	1.15	28.5
A 10	1.01	1.18	0.93	432.6	1.50	27.0
A 15	1.02	0.84	0.88	408.3	1.35	27.5
B 6	1.00	1.23	0.95	399.1	1.63	28.9
B 10	1.04	1.14	0.95	406.3	1.37	28.0
B 15	1.04	1.19	0.9	408.5	1.10	28.3
V 7	0.93	1.09	0.94	388.1	1.23	27.9
V 9	0.92	1.2	0.88	443.1	1.70	27.6
26	0.96	1.12	0.98	394.5	1.30	27.8
31	1.07	1.19	0.96	361.1	1.75	27.9

* Clone = 2-3 trees (ramets); tree = 4 specimens

Discussion

- Acetone extractives **2.22%** in *Picea abies* (Fengel & Wegener, 2003, Wood)
- EN 350-2: Density and natural durability of some wood species against fungi

Species	Origin	Density/ range of mean values at 12% (m/m), kg/m ³	Durability class
<i>Picea abies</i>	Europe	440- <u>460</u> -470	4 (slightly durable)
<i>Pinus sylvestris</i>	Europe	500-520-540	3 – 4 (moderately durable-..)
<i>Pinus contorta</i>	N. America	430-460-470	3 - 4
<i>Quercus robur</i>	Europe	670- <u>710</u> -760	2 (durable)

Durability – heartwood only, sapwood – all species class 5 (not durable)



CONCLUSIONS

- No clear relationship among the wood decay, wood density, content of extractives and lignin.
- Juvenile wood prevailed.
- Wood contained more sapwood with lower density and content of extractives.
- Use for interior structures; for outdoor structures -additional protection necessary.
- Recommended for manufacturing of wood based products (medium density fiberboards etc.), furniture or pulp and paper.



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Thank you!

**Study carried out in European Social Fund's Project
"Importance of Genetic Factors in Formation of Forest
Stands with High Adaptability and Qualitative Wood
Properties" (No 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146)**

Within stand relatedness and genetic diversity

Tiit Maaten

We have a dream....

to know all sequences by all trees
what we have and their relations
with all traits.

Why to study?

- For determining stand or population structure and diversity
- For evaluating population viability
- For having material for evaluation the influence of breeding on diversity/relatedness
- For determining origin of forest trees
- For any other purpose which could help us understand how trees are behave and use such information by silvicultural or conservation actions

Such information is very interesting but...

- We are not able to get it
- We must use less sources for getting such information
- If the purpose will be investigation of single stand (or few stands) then how we could define a term “stand”?
- Do we have enough finances for getting that?



What is a stand in this case?

- There are many tree species on good site types: number of target individuals per ha could be low
- How old the stand must be? Can we consider two years old stand as a stand?
- Shall we consider some number of trees of the target species as one unit stand? Some average number of effective population size?



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Background – how to get money? CBD

- **CBD article 2: "Biological diversity"** means the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this **includes diversity within species**, between species and of ecosystems.
- **Article 6. General measures:** Each Contracting Party shall, in accordance with its particular conditions and capabilities: (a) Develop national strategies, plans or programmes for the conservation and sustainable use of biological diversity or adapt for this purpose existing strategies, plans or programmes which shall reflect, inter alia, the measures set out in this Convention relevant to the Contracting Party concerned; and (b) **Integrate**, as far as possible and as appropriate, the conservation and sustainable use of **biological diversity into relevant sectoral or cross-sectoral plans, programmes and policies**.
- **CBD article 12: Research and Training:** The Contracting Parties, taking into account the special needs of developing countries, shall: b) Promote and encourage research which contributes to the conservation and **sustainable use of biological diversity**, particularly in developing countries, inter alia, in accordance with decisions of the Conference of the Parties taken in consequence of recommendations of the Subsidiary Body on Scientific, Technical and Technological Advice;



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Background – how to get money? MCPFE

- **S2:** considering that, above and beyond the conservation of forest species, the essential objective is the conservation of the genetic diversity of these species, which are an essential part of mankind's heritage.
- **S2:** recognizing, also that the use of genetically-improved materials is of great importance for afforestation and restocking, in particular where this is for the purpose of the production of timber.
- **H4:** (Areas for research) Studies on genetic variability of regionally important tree species in response to changes in climate and increased concentration of carbon dioxide, and on the degree and rate of evolutionary processes and adaptation, by means of genetic changes.

Strasbourg 2 resolution:

- Research for conservation
- Research for breeding (silviculture)

Conservation of FGR in Europe

- EUFGIS database available www.eufgis.org
- It was compiled on the basis of minimum requirements for gene conservation units



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GCU-s

The units should have a designated status as gene conservation areas of forest trees at national level. The units can be located in forests managed for multiple uses, protected areas or seed stands. The minimum size of a unit depends on tree species and conservation objectives as follows;

- 1) **500 or more reproducing trees** (when the objective is to **conserve gene diversity** of widely occurring and stand-forming conifers or broadleaf species),
- 2) **50 reproducing trees** (when the objective is to **conserve adaptive or other traits in marginal or scattered tree populations**) or **50 seed bearing trees** (scattered tree species with sexual dimorphism), and
- 3) **15 unrelated reproducing trees** (when the objective is to **conserve remaining populations of rare or endangered tree species**).

In light of presented minimum requirements, we are facing the same problem: how define a stand and how many samples do we need for determination the genetic structure and level of relatedness by single stand

Short overview about literature

Papers available

- Common fashion by papers: 5-40 samples per stand or per population analysed, different variables calculated
- In some cases all trees analysed, usually in case where stands are very small and/or rare, especially in the Mediterranean region
- One paper where bigger number of forest trees per one stand analysed (Chybicki *et al.*, 2008).

One problem:

Genome size (bp):	No of genes,	% of coding DNA
<i>Escherichia coli</i> - $4,7 \times 10^6$	4 000	100
<i>Arabidopsis th.</i> - 125×10^6	25 500	50
<i>Picea abies</i> - $30\,000 \times 10^6$	25 000 – 50 000	<3
<i>Fritillaria</i> sp. - $85\,000 \times 10^6$	25 000	0,02
<i>Homo sapiens</i> - $3\,000 \times 10^6$	40 000	<2

Spatial Genetic Structure Within Two Contrasting Stands of Scots Pine (*Pinus sylvestris* L.)

The spatial genetic structure of two contrasting stands of Scots pine located within one continuous population investigated: (i) the even aged qualified seed-tree stand utilized for commercial seed collections (321 trees) and (ii) the naturally established unevenaged and unmanaged stand (173 trees).

Genetic variation at 14 allozyme loci (*Fest*, *Gdh*, *Aat-1*, *Aat-2*, *Aat-3*, *Mdb-1*, *Mdb-3*, *Mdb-4*, *6Pgdb-1*, *6Pgdb-2*, *Pgi-2*, *Pgm-1* and *Shdb-1*) studied

Two stands differed substantially in the levels of inbreeding. The estimated *FST* coefficients also showed that the two studied stands genetically are not very differentiated at the single locus level (although at the multilocus genotypic level they are)

In spite of such strong differences there were only slight differences either in genetic diversity and spatial genetic structure.

Common conclusion by investigations of Scots pine populations

Despite periodic interstadial fragmentation episodes, Scots pine biology provides for the long –term maintenance of high within-population and low among-population genetic diversity at neutral genetic markers (Robledo-Arnuncio *et al.*, 2005)

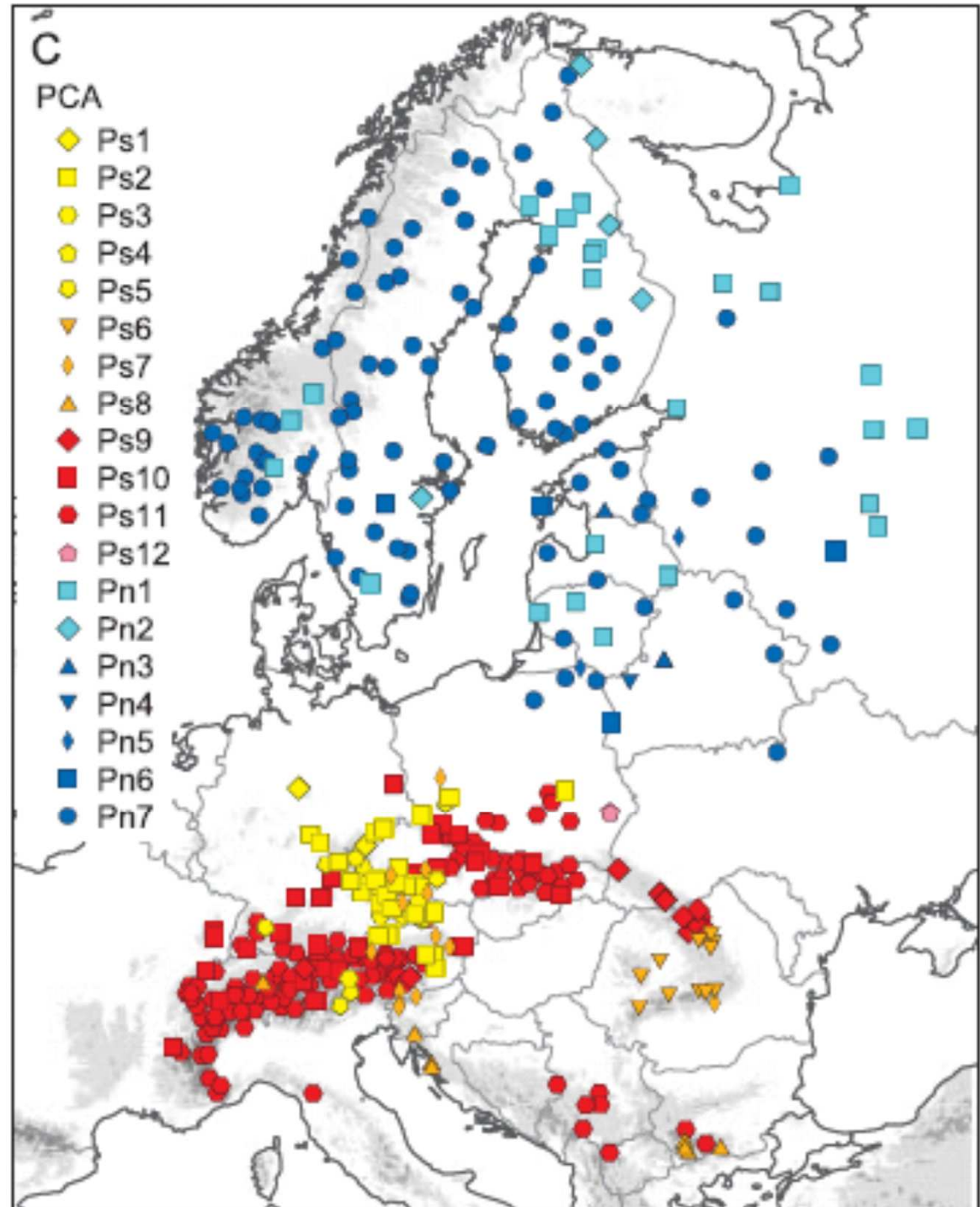
This conclusion was made on the basis of cpSSR analyses

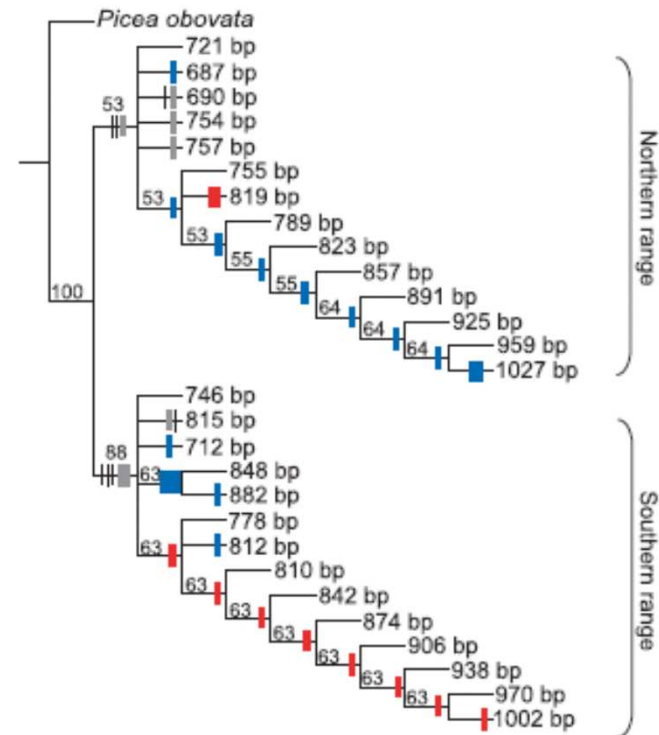
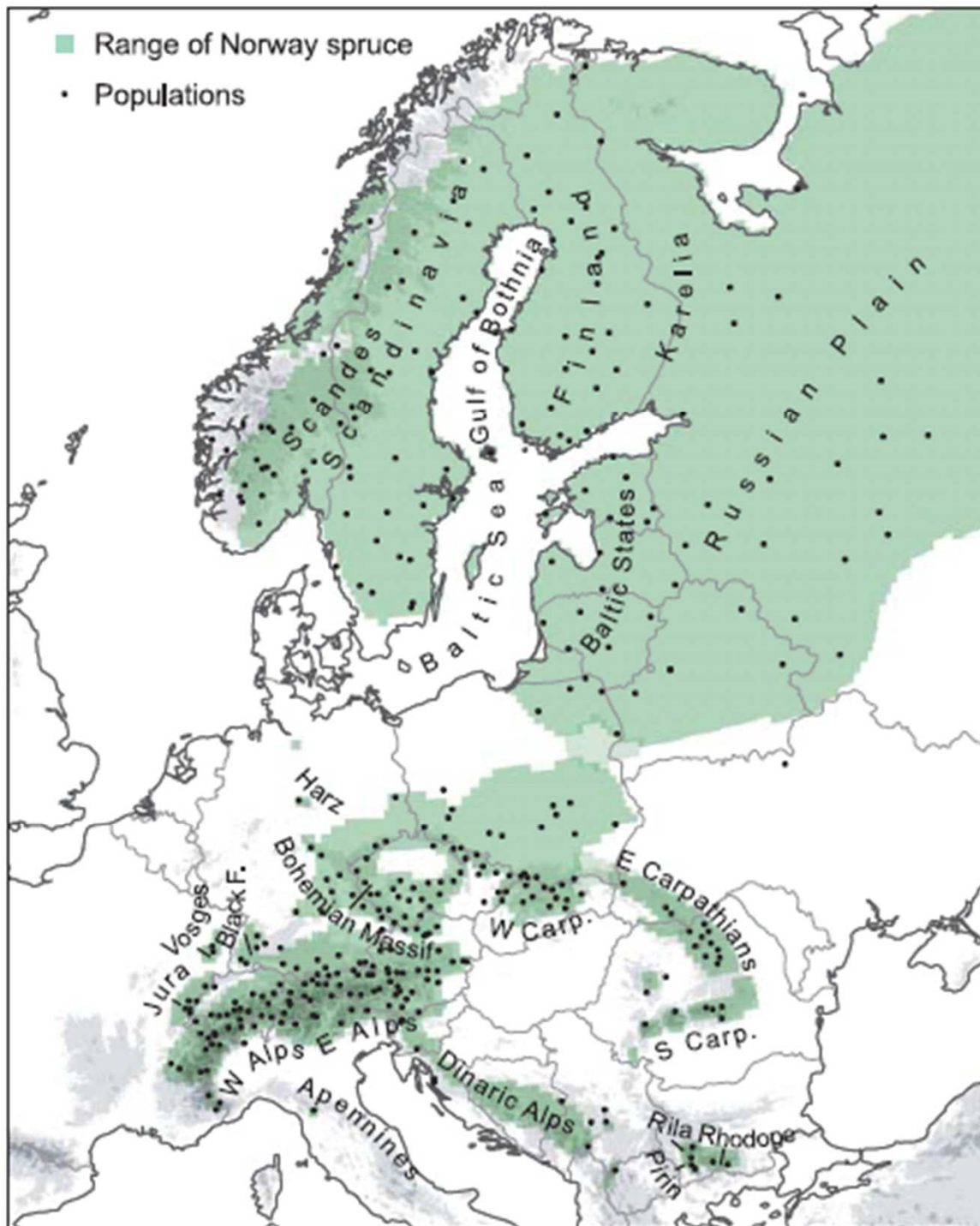


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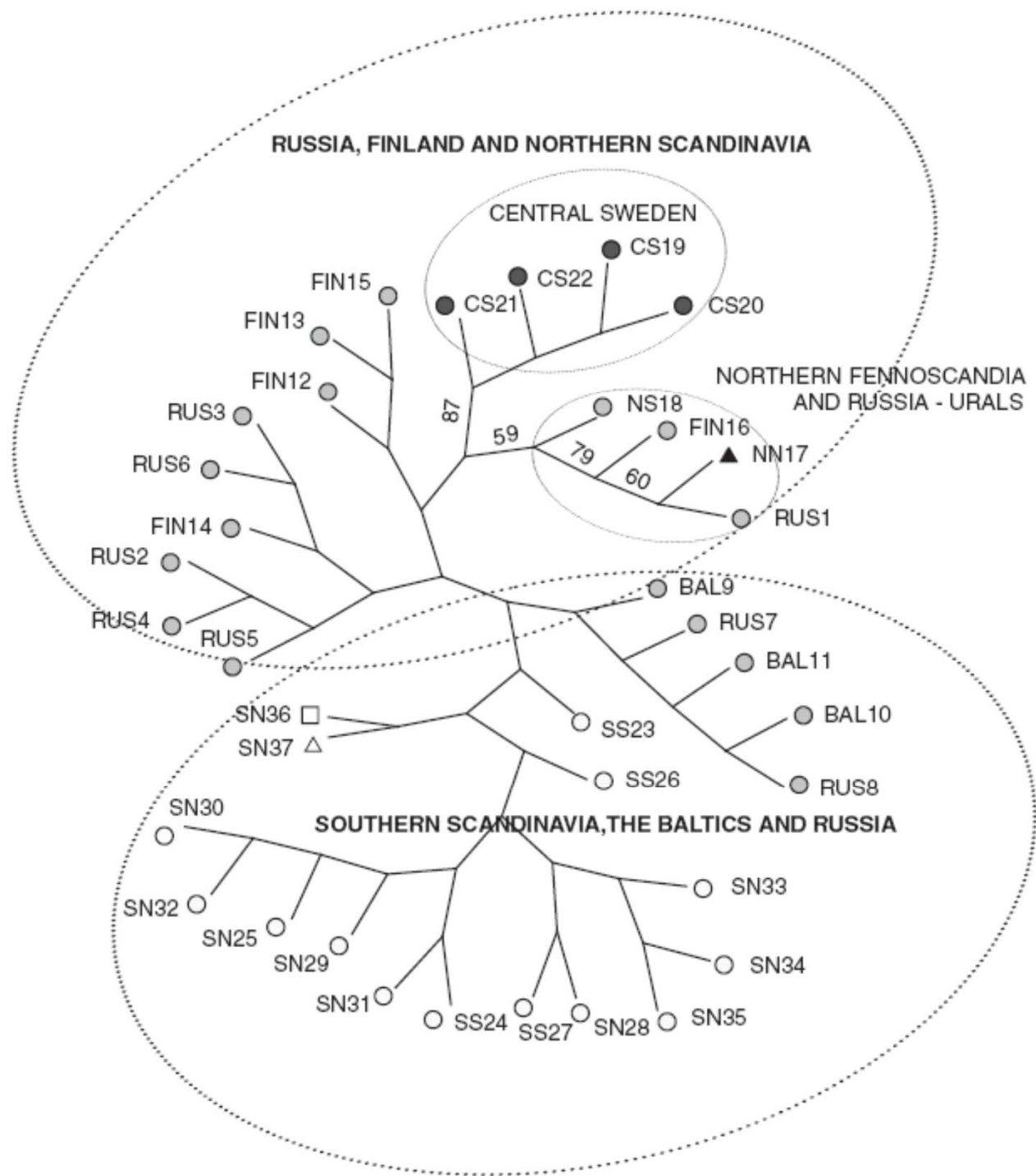
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Tollefsrud *et al.*, 2008.
Genetic consequences
of glacial survival and
postglacial colonization
in Norway spruce:
combined analysis of
mitochondrial DNA
and fossil pollen –
Molecular Ecology, 17,
4134 - 4150





Tollefsrud *et al.*, 2008. Genetic consequences of glacial survival and postglacial colonization in Norway spruce: combined analysis of mitochondrial DNA and fossil pollen – *Molecular Ecology*, 17, 4134 - 4150



Combined analysis of nuclear and mitochondrial markers provide new insight into the genetic structure of North European *Picea abies* (Tollefsrud et al. 2009 (Heredity))



Thanks!

European Social Fond project (No.
2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146)



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Wood trait differences of hybrid aspen clones

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Materials and method



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Sample trees cut at age 12, in 2 hybrid aspen clones experimental trial, and addition sample trees cut in another 2 experiments.

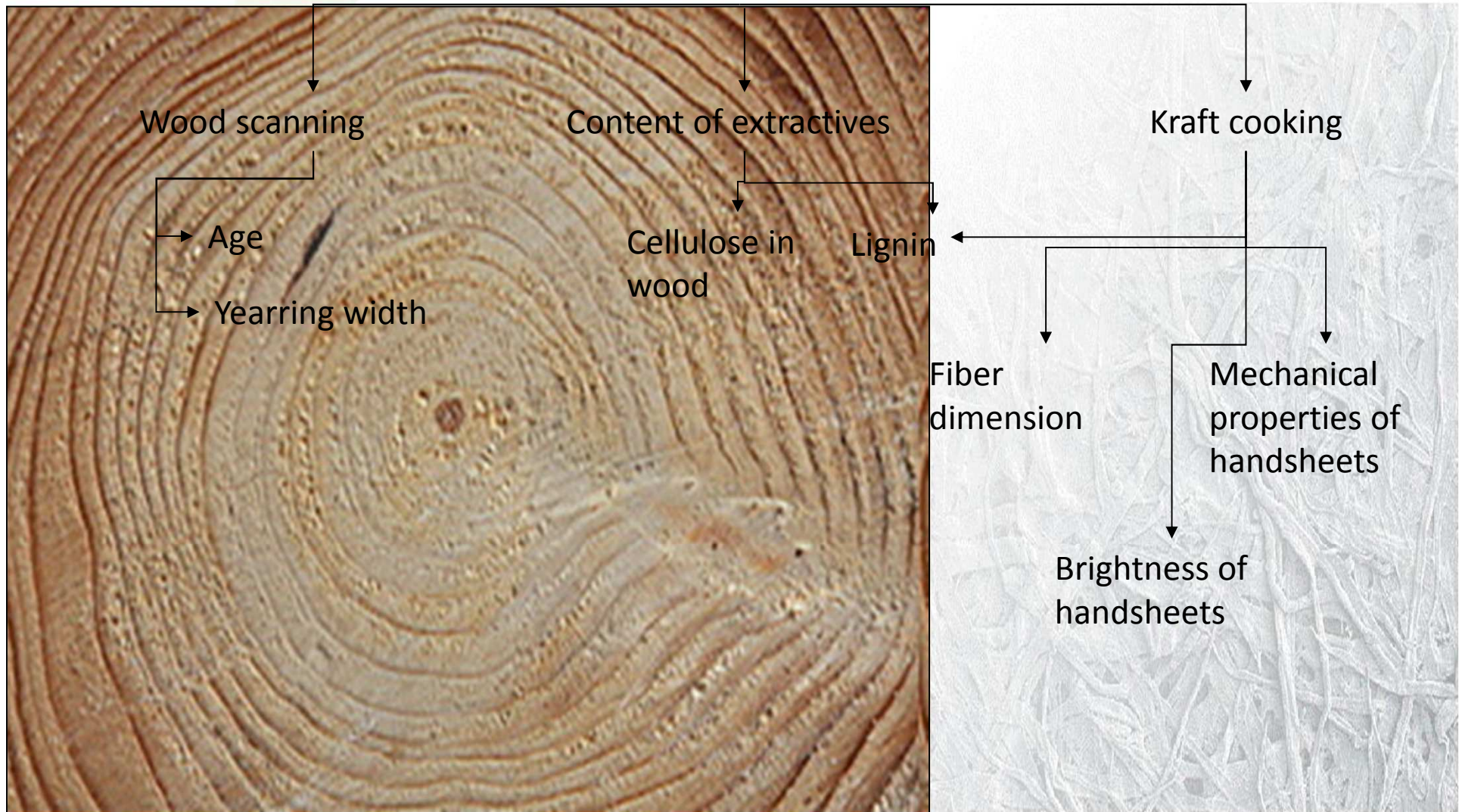
Wood used for analysis of the trunk from 0.5 to 1.3 meters in height from the ground.



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Materials and method

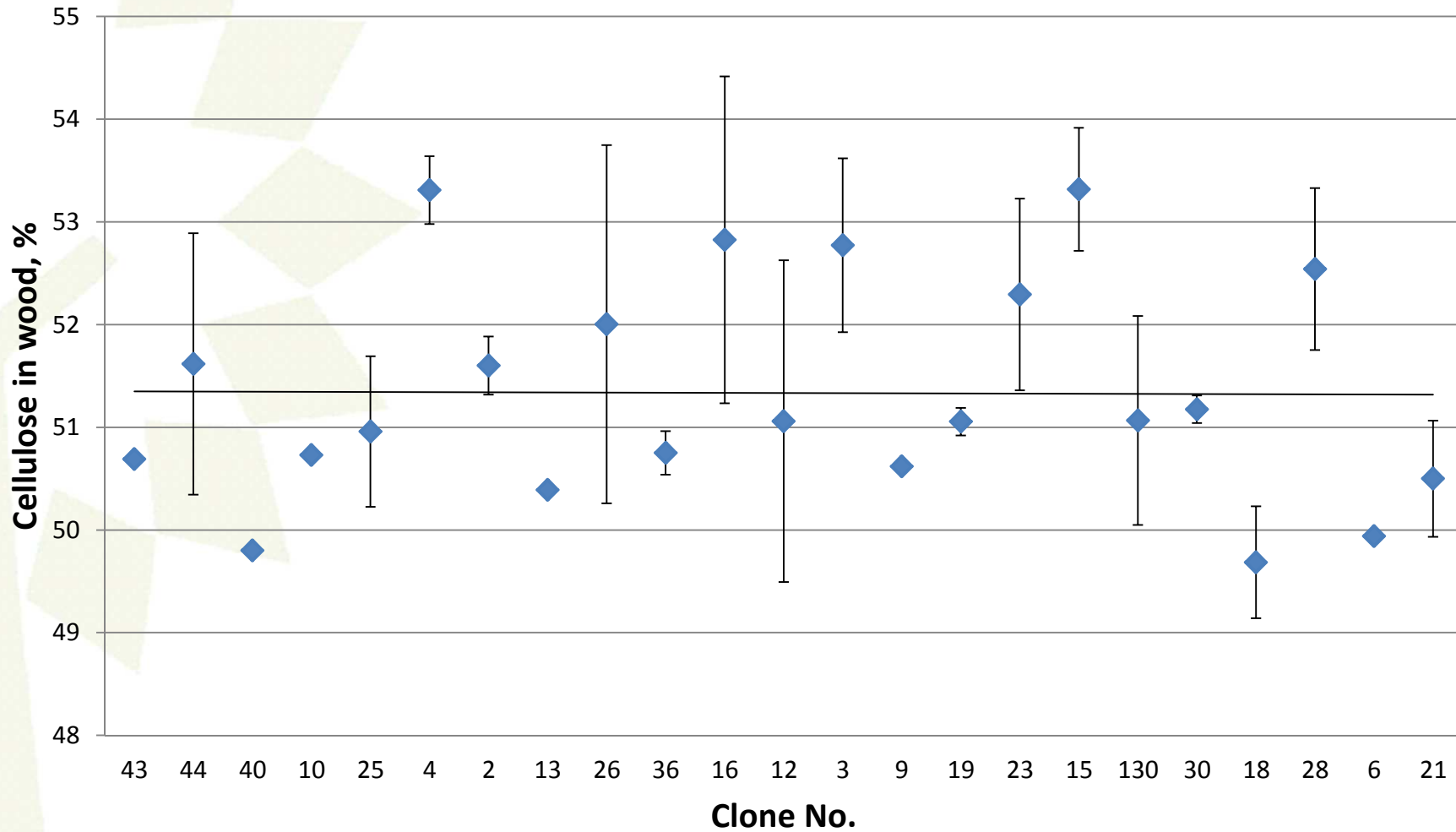




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Results of Cellulose in wood, %

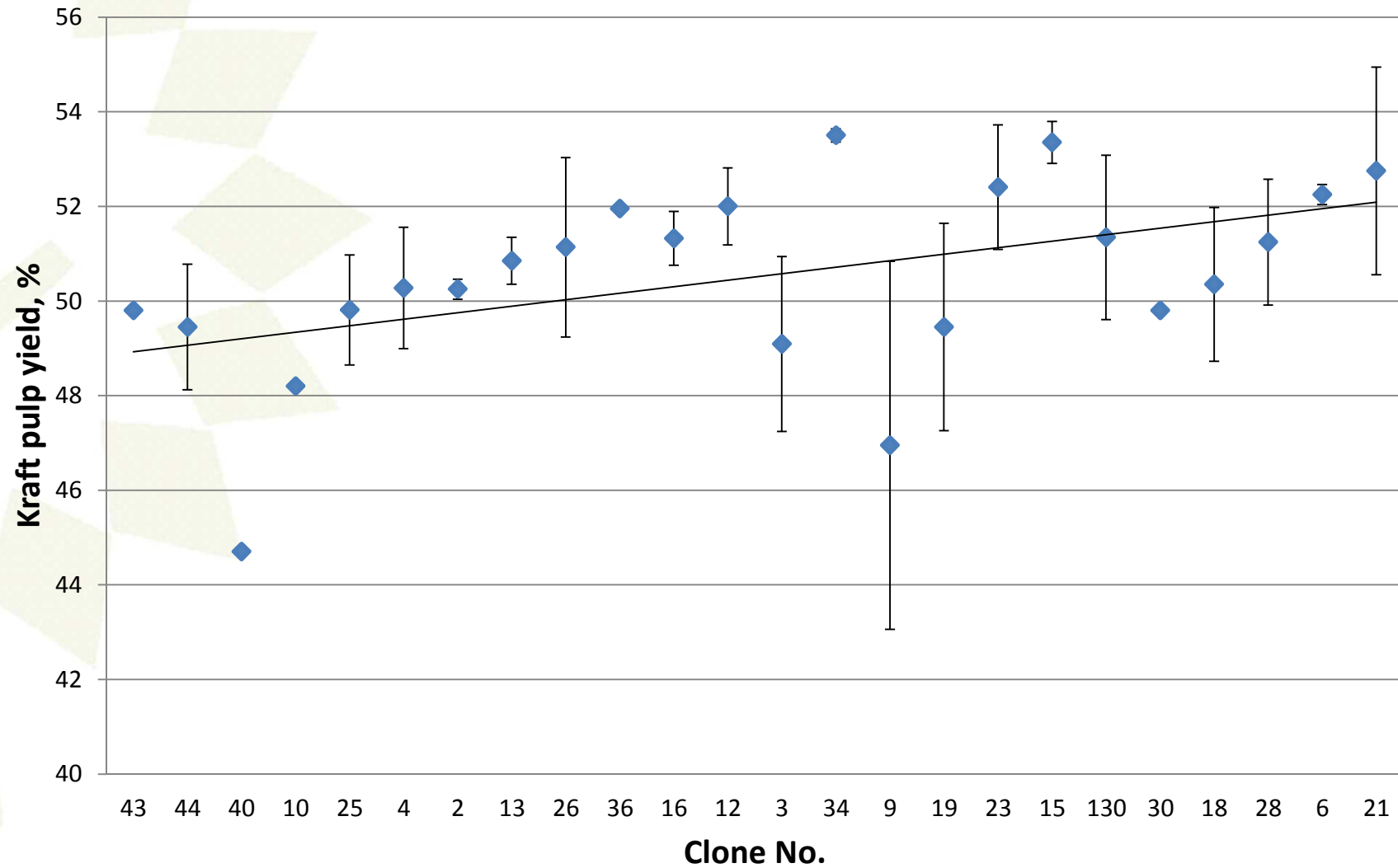




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Results of Kraft pulp Yield %





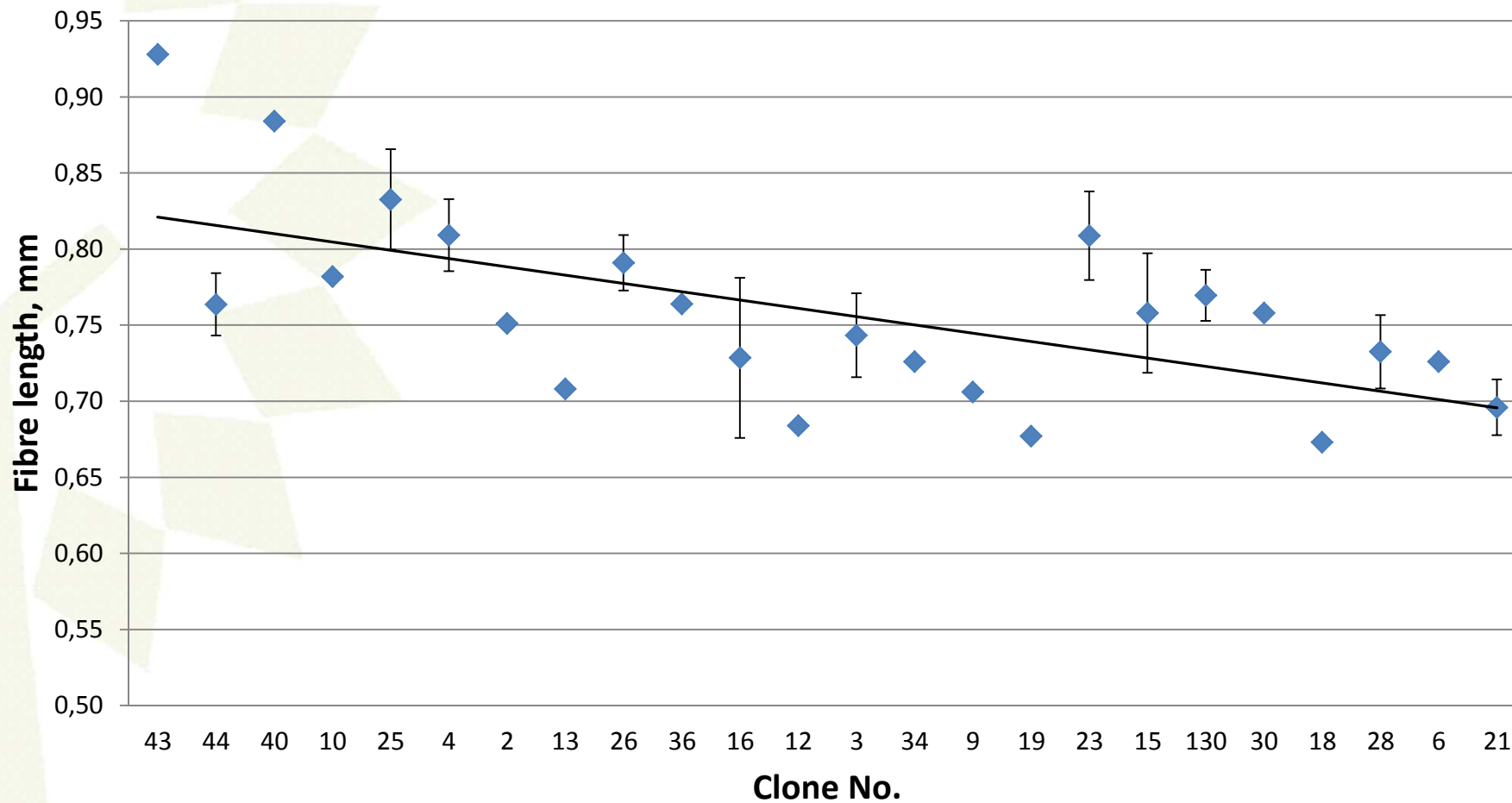
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Results of Fibre length, mm



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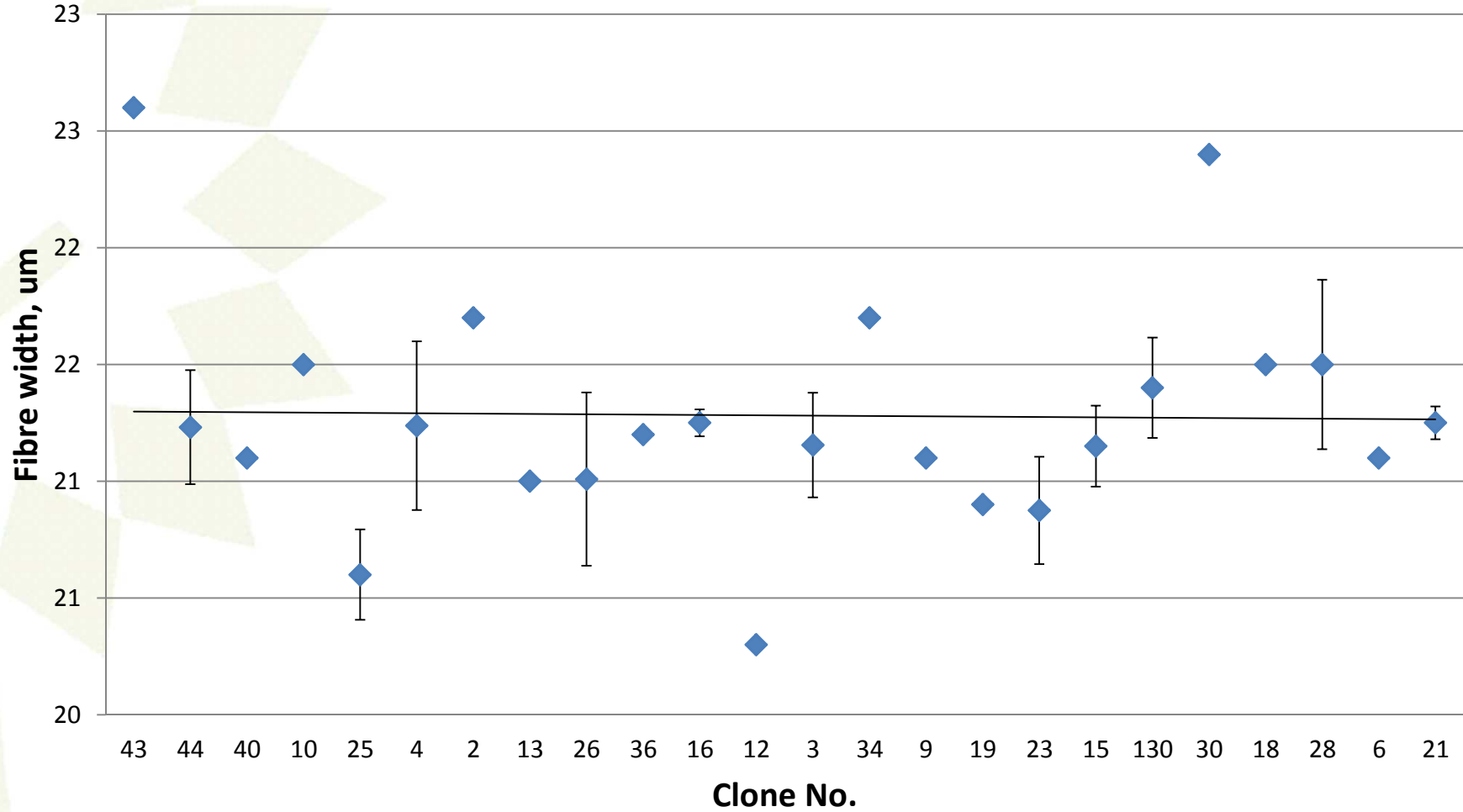




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Results of Fibre width, μm





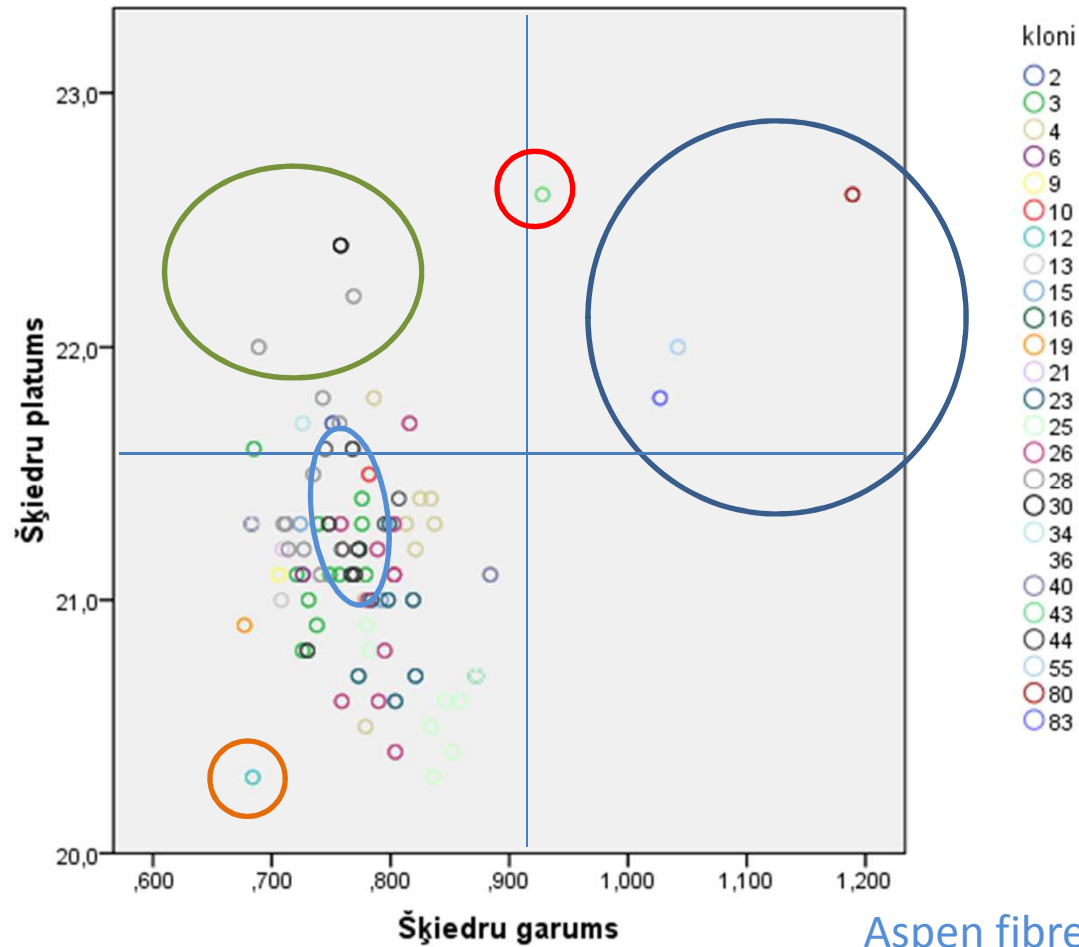
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Results of Fibre length and width



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Aspen fibres region

Old aspen hybrids (~40y)

43 to present highest length and width

12 presents shorter and more arrow fibres

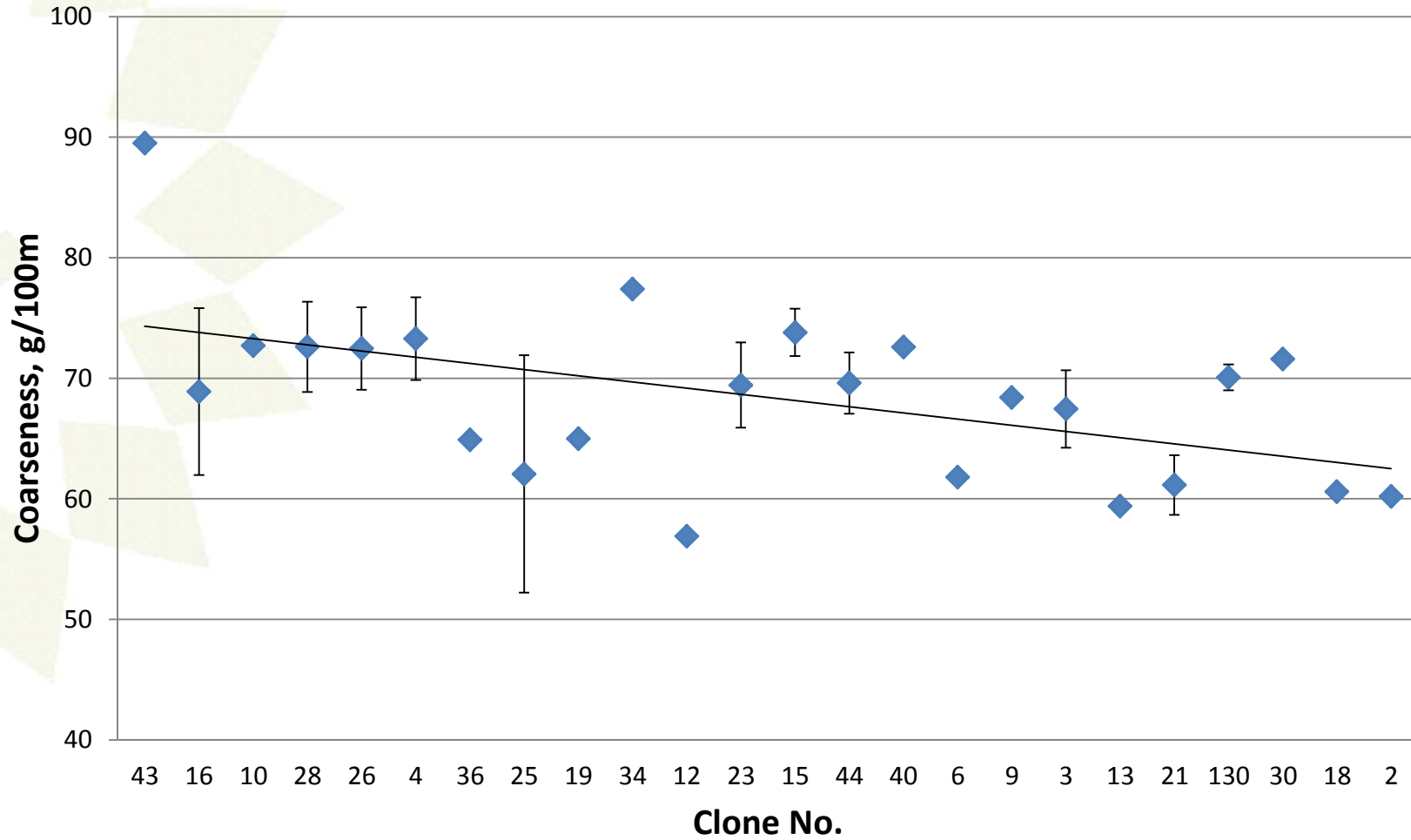
28, 30 are presented by "shorter" and more wider fibres



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Results of Coarseness, g/100m

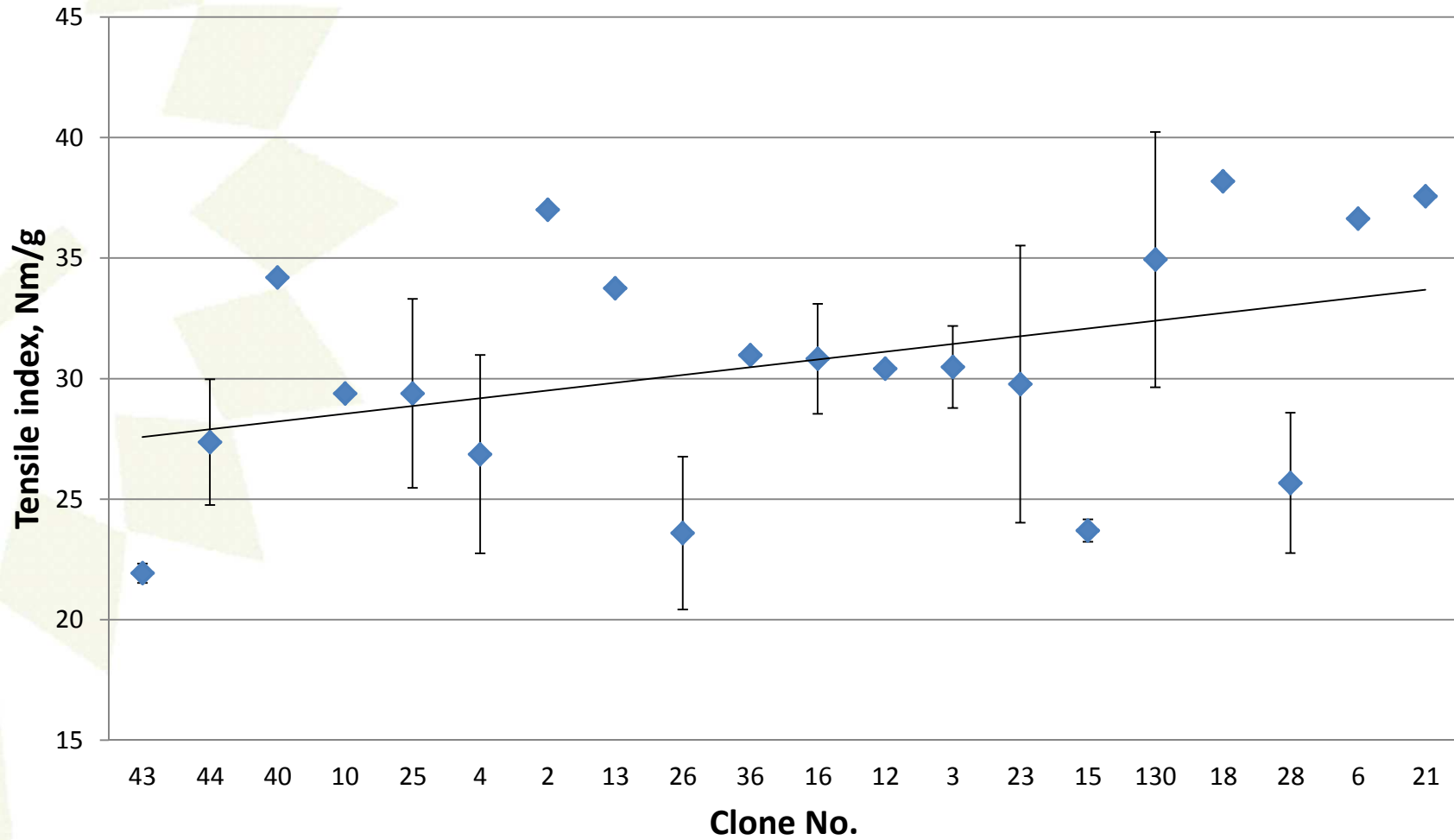




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Results of Tensile index, Nm/g





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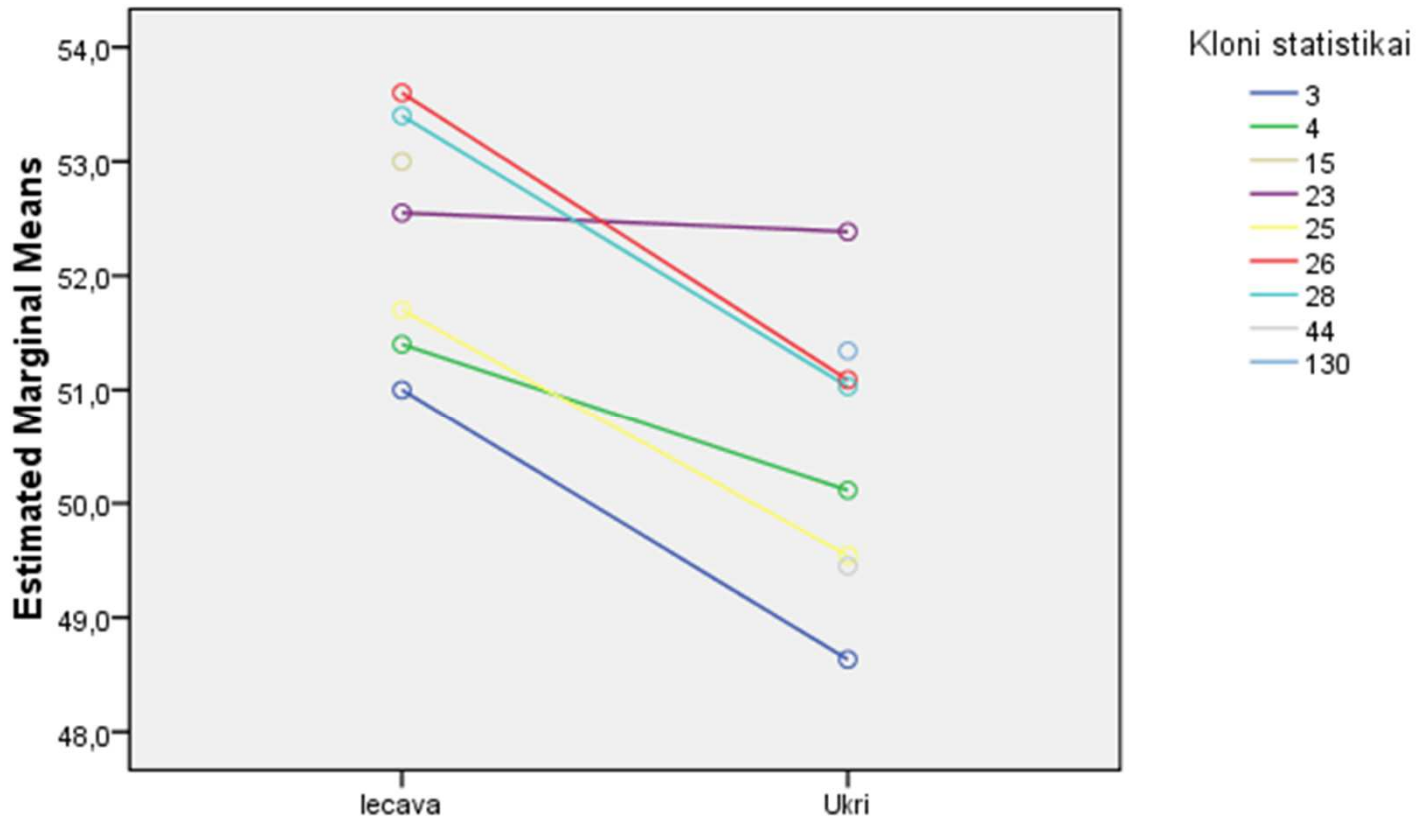
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Best clones for pulp un paper plantations is 23

Plantation region makes effect on Kraft pulp yield of Aspen (130); 25, 26 and 28 hybrid aspen clones, but no so much effect on hybrid clone No.4.





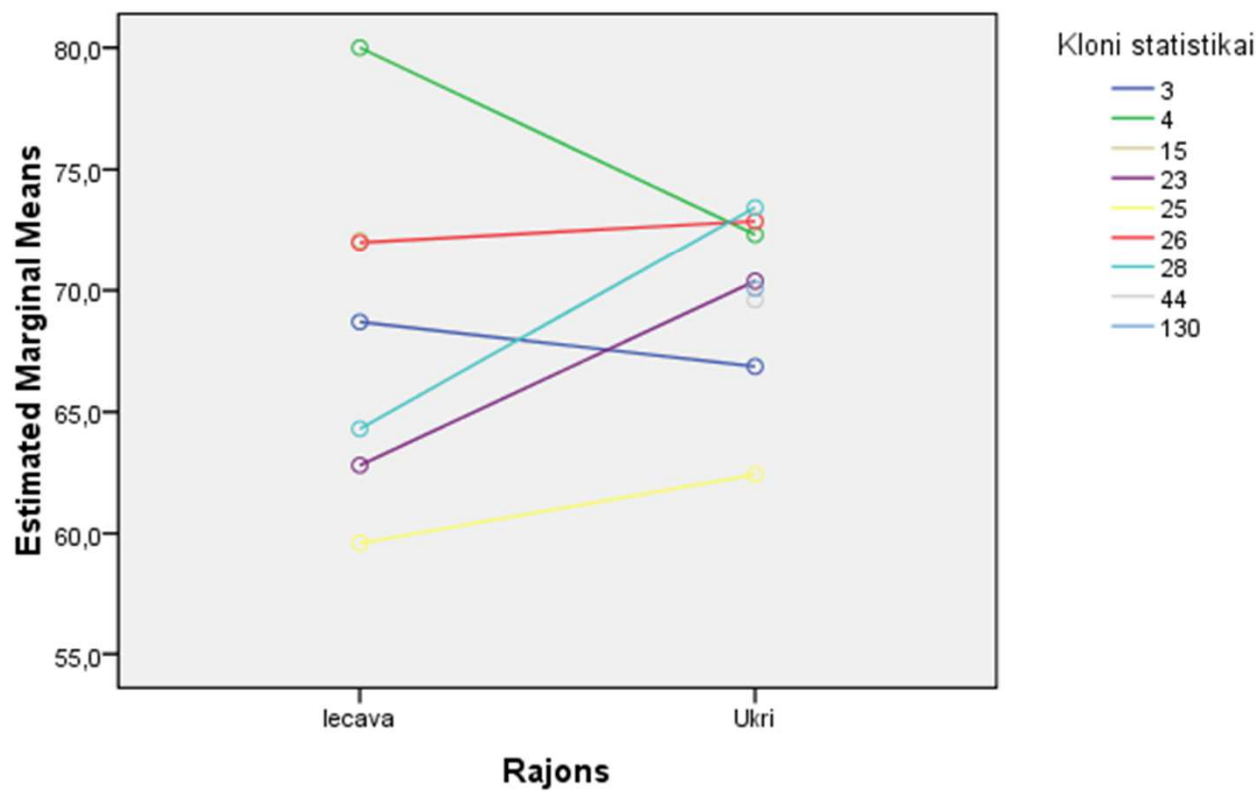
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Estimated Marginal Means of Raupjums



Non-estimable means are not plotted



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Results



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1. It is possibilities to select hybrid aspen clons for polp and paper production. Perspective clones for pulp and peper industry is 23, 43, 4 , 28, 40.
2. Hybrid aspen clone 23 is stable in different plantations.
3. Hybrid aspen clones have more cellulose content in wood comparison this common aspen. For industrial purposes is it essentially.



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Thank You for attention!



SILAVA

Study has been partly funded by:

ESF project “Importance of Genetic Factors in Formation of Forest Stands with High Adaptability and Qualitative Wood Properties”
(No 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146)

