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Rooting Acacia mangium Cuttings: Effects of Age, Within-Shoot Position and Auxin Treatment

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Summary

The possibilities of vegetatively propagating *Acacia mangium* through rooted cuttings were examined, focusing on 3 main factors, namely: (i) the age of the donor plant, comparing 6-month-old seedlings to sprouts from a mature stump, (ii) the original within-shoot position of the cutting before collection and (iii) the auxin treatment applied to the cuttings. These 3 factors were shown to influence greatly the capacity of the cuttings for rooting. The best scores in terms of rooting rates were obtained for the plant material collected from (i) the seedlings, (ii) the upper part of the shoot close to the terminal bud and (iii) when treated with auxin. The only interaction confirmed by statistical tests was between the age of the donor plant and the auxin treatment, with a greater auxin induced increment of the rooting rates for the cuttings from seedlings.

The results obtained tend to demonstrate that, although capable of improvement by optimizing the investigated factors, the potential of *Acacia mangium* to be propagated by rooted cuttings remains rather limited, especially when starting from mature material.

Key words: Acacia mangium, adventitious rooting, auxin, cuttings, maturation, vegetative propagation, within-shoot position.

FDC: 165.44; 161.4; 232.5; 176.1 *Acacia mangium.*

Introduction

Acacia mangium WILLD., an arborescent species belonging to Leguminosae (Mimosoideae), is native to northern Australia (Queensland), western parts of Papua New Guinea and eastern provinces of Indonesia (Irian Jaya and Maluku) from sea level up to 720 m (GUNN and MIDGLEY, 1991). It has been recognized as a valuable forest tree species since its initial introduction into Sabah (Malaysia) in 1966, where it has been demonstrated to thrive quite well, even on very acid and infertile soils whose fertility can be restored thanks to its natural nitrogen fixing ability. In such poor but rather frequent site conditions, it has been shown to outperform all the other fast growing forest tree species, especially in situations of proliferating weeds with which it competes successfully. The early vigorous growth of this short-lived pioneer enables it to reach commonly 20 m to 25 m in height within 10 years to 15 years in Sabah, with a wood production averaging 25 m^3/ha/year to 30 m^3/ha/year, despite the mediocre genetic value of the material planted so far (SIM, 1986). Owing to this remarkable potential, which accounts for the establishment of industrial plantations all over South-East Asia mainly for pulpwood production, it appears obvious as a matter of urgency to concentrate efforts on tree improvement of *A. mangium* with a view of improving the genetic quality of the planting stock. Progress in that field is just beginning and although simple breeding strategies based on sexual propagation seem objectively well adapted to the situation (MONTEUUIS and NASI, 1992), the vegetative propagation option is also worth special attention. Theoretically, it can be used for mass "bulk" vegetative propagation of a restricted number of juvenile genotypes of presumably high genetic value — derived for example from controlled pollinations — (WONG and HAINES, 1992), or for producing clones, either to establish clonal seed-orchards, or to develop clonal plantations, especially from genotypes selected from clonal tests of advanced generation families

(HAINES and GRIFFIN, 1992). However, although highly desirable in theory, the prospects of applying vegetative propagation to *A. mangium* are dependent on the ability of this species to be propagated by rooted cuttings.

So far, little information is available regarding this matter. However, it appears from previous studies (DARUS, 1991; HAINES et al., 1991) that the age of the plant material is likely to play a determining role, as with other arborescent species where the maturation process has been observed to act negatively on the potential for adventitious rooting (BONGA 1982; HACKETT, 1988). Another factor that should warrant special consideration according to WONG and HAINES (1991) is the initial position of the cutting within the shoot of the ortet — the donor plant it has been collected from — and distinguishing between the terminal-shoot cuttings and the single-node ones. The same authors reported also the beneficial effect of treating *A. mangium* cuttings with root-promoting exogenous substances, the so-called "auxins", specifically "Seradix 3", also used by DARUS (1991), to enhance the quantity and the rate of production of adventitious roots.

The influence as well as the possible interaction of these three factors on the rooting potential of *Acacia mangium* stem cuttings have been assessed.

Material and Methods

Softwood cuttings of *A. mangium* used for the rooting experiments were collected from 2 different donor plant types:

a. main stems of 6 month-old seedlings cultivated in the nursery;

b. 2 month-old sprouting shoots emerging from the stump of a 6 year-old ortet growing near the nursery.

The average size of the cuttings ranged between 6 cm to 8 cm in length and 3 mm to 5 mm in diameter. Except for the terminal-shoot with its apical bud, they consisted of one single node plus the full internode underneath. About half of the surface of the phyllodes — leaf-like petioles — was removed in order to lower evapotranspiration and to reduce water stress risks.

The original position of each cutting within the shoot was noted according to a basipetal numeration, with the terminal shoot cutting as number 1, the nodal segment cutting just below it as number 2, and so on down to number 8.

Half of the cuttings corresponding to each of the different origins used were treated with auxin by dipping their base into the Seradix 3 commercial powder preparation (0.8% of 3-indolebutyric acid in talc), whereas the remaining half was not treated to serve as control.

The next step consisted of inserting all the cuttings into the rooting beds filled with wet sand used as rooting substrate after it had been boiled with a view of reducing disease risks.

The experimental design adopted corresponded to a full factorial of (i) the age of the donor plant (2 classes); (ii) the within-shoot position of the cuttings (8 classes) and (iii) the auxin treatment (2 classes), resulting in a total of 2 x 8 x 2 = 32 combinations, each being represented by an experimental plot of 10 totally randomized cuttings. Three complete blocks of this type were set up applying rigorously the same procedure on the following dates and under the same equatorial humid climatic conditions:

— 1st block on the 24th of April 1992;
— 2nd block on the 22nd of May 1992;
— 3rd block on the 23rd of June 1992.

To sum up, the whole experiment included:
10 x 2 x 8 x 2 x 3 = 960 cuttings.

Once set into the rooting bed, the cuttings were maintained under a 50 % shade with intermittent-mist water sprays provided by a mist system, the frequency of which was controlled by the "electronic leaf" system (HARTMANN et al., 1990) to avoid any desiccation damage. Aqueous fungicide solutions — mainly Thyram 80, 5 g/l — were sprayed on the cuttings once a week.

After a rooting period of 2.5 months in these conditions, the cuttings were assessed by recording the following criteria:

1. rooting rate for cuttings (RRC), based on the 10-cutting plots;
2. number of adventitious roots per rooted cutting;
3. root score, defined as the variation coefficient of 3 values corresponding to the number of newformed roots in each axial third of the cross-sectional area of each rooted cutting; thus, the lower the root score, the more even the root distribution around the cutting axis;
4. length of the longest root (in cm) per rooted cutting.

The data were analyzed using the SAS statistical package (SAS Institute Inc., 1988). The null hypotheses were rejected when probability value $P \leq 0.050$. Tests for homogeneity of variance were performed using BARTLETT's test (SNEDECOR and COCHRAN, 1957) and LEVENE's test (TOMASSONE et al., 1983) which both established that the computed variances of RRC were not homogenous enough when referring to classes within each factor, especially regarding the age of the donor plant ($P \leq 0.001$). For this reason RRC was suitably replaced by ASRRCC according to the following angular transformation (SNEDECOR and COCHRAN, 1957): ASRRC = 2 $\arcsin\sqrt{\text{RRC}}$.

The effects of the experimental factors on the ASRRC values were evaluated carrying out an analysis of variance according to the following statistical model:

$$Y_{ijkl} = \mu + A_i + P_j + H_k + B_l + (AP)_{ij} + (AH)_{ik} + (PH)_{jk} + (APH)_{ijk} + \varepsilon_{ijkl}$$

where:

Y_{ijkl} = value of ASRRC of the plot submitted to the i^{th} level of factor age of the donor plant, j^{th} level of factor within-shoot position, k^{th} level of factor auxin in the l^{th} block;
μ = overall mean of the trial;
A_i = effect of the factor age of the donor plant, $1 \leq i \leq 2$;
P_j = effect of the factor within-shoot position, $1 \leq j \leq 8$;
H_k = effect of the factor auxin, $1 \leq k \leq 2$;
B_l = effect of blocks, $1 \leq l \leq 3$;
$(AP)_{ij}$ = effect of the interaction of age and within-shoot position;
$(AH)_{ik}$ = effect of the interaction of age and auxin;
$(PH)_{jk}$ = effect of the interaction of within-shoot position and auxin;
$(APH)_{ijk}$ = effect of the interaction of age, within-shoot position and auxin;
ε_{ijkl} = random error.

Deficiency in the ability to form adventitious roots resulted in a large number of missing values especially for the cuttings taken from the lower positions within the shoot. For this reason, the statistical analyses for the

Table 1. — Mean values of the 4 characters assessed for each experimental factor investigated Letters distinguish means which are significantly different at the 5% level. Data in brackets were not submitted to the analyses of variance and covariance and must be considered as informative only.

CHARACTERS	RRC1 (%)		No of plots6	No of roots2		Score3		Length4 (cm)		No of plots
FACTORS	mean	σ5		mean	σ	mean	σ	mean	σ	
BLOCK										
1	27.2	27.3	32	2.3	0.7	0.78	0.33	10.0*ab*	2.5	11
2	28.8	29.4	32	2.5	1.4	0.85	0.45	11.7 *a*	3.1	12
3	28.1	30.6	32	2.1	0.7	0.88	0.37	7.5 *b*	1.9	10
DONOR PLANT										
Seedlings	41.5 *a*	30.6	48	2.2	1.0	0.88	0.26	9.7	2.4	18
Stump	14.6 *b*	19.3	48	2.4	1.0	0.79	0.50	10.1	3.7	15
WITHIN-SHOOT POSITION										
1	59.2 *a*	26.8	12	3.0 *a*	1.0	0.60	0.32	9.5	2.0	12
2	45.0*ab*	26.1	12	2.0*ab*	0.8	1.00	0.28	10.3	4.0	11
3	30.8*bc*	25.4	12	1.8 *b*	0.7	0.94	0.41	9.9	3.1	10
4	22.5*cd*	21.8	12	(2.0)	(1.2)	(1.00)	(0.37)	(9.9)	(2.9)	(9)
5	20.8*cd*	23.5	12	(2.3)	(0.9)	(0.98)	(0.27)	(10.9)	(2.7)	(8)
6	22.5*cd*	33.1	12	(1.5)	(0.9)	(1.27)	(0.25)	(8.6)	(2.6)	(7)
7	12.5 *d*	27.0	12	(2.1)	(0.4)	(0.79)	(0.19)	(5.6)	(1.6)	(3)
8	10.8 *d*	13.8	12	(2.0)	(0.7)	(0.79)	(0.51)	(8.1)	(3.8)	(6)
AUXIN										
Control	17.5 *a*	23.7	48	1.9 *a*	0.7	0.92	0.47	9.5	2.3	15
Seradix 3	38.5 *b*	29.9	48	2.7 *b*	1.0	0.77	0.50	10.2	3.6	18
OVERALL SCORES	**28.0**	**28.8**	**96**	**2.3**	**1.0**	**0.84**	**0.38**	**9.9**	**3.0**	**33**

1: Rate of rooted cuttings.
2: Number of roots per rooted cutting.
3: Root score.
4: Length of the longest root per rooted cutting.
5: Standard deviation.
6: Number of elementary plots; initially every elementary plot included 10-cuttings.

three characters of the newly formed root system were performed only for the cuttings originating from the three upper within-shoot positions. The same analysis of variance model as for ASRRC was used for the number of adventitious roots and the length of the longest root, whilst the root score was submitted to an analysis of covariance with the number of roots as covariable due to the high correlation existing between these 2 variables. The relevant model was:

$$Y_{ijkl} = \mu + A_i + P_j + H_k + B_l + (AP)_{ij} + (AH)_{ik} + (PH)_{jk} + (APH)_{ijk} + \beta (X_{ijkl} - \bar{X}_{....}) + \varepsilon_{ijkl}$$

where μ, A_i, P_j, H_k, B_l, $(AP)_{ij}$, $(AH)_{ik}$, $(PH)_{jk}$, $(APH)'_{ijk}$, ε_{ijkl} as previously defined and in addition:

β = the true common slope of the regression lines;
X_{ijkl} = root number value concomitant to Y_{ijkl};
$\bar{X}_{....}$ = overall average of the root number covariable.

Treatment means were compared using STUDENT-NEWMAN and KEULS test when in F-tests the null hypothesis was rejected ($P \leq 0.05$).

PEARSON's correlation coefficients between the 4 variables, RRC being replaced by ASRRC, were calculated computing the data from plots including at least one rooted cutting, that is to say in 66 cases out of 96.

Results

General outlines

At the end of the 2.5 month rooting period, 269 cuttings were found rooted out of the total of 960 initially set, which corresponded to an overall rooting rate of 28 %. Among the unrooted cuttings, very few remained alive (15 out of 691). The highest rooting rate of 80 % (24/30) over the 3 dates ("blocks") was obtained for cuttings originating from the second within-shoot position of the seedlings and treated with Seradix 3. The lowest rooting rates (0 %) corresponded in most cases to experimental combinations involving cuttings from the base of sprouts from the stump. *Table 1* presents the mean values of the various characters assessed for each experimental factor.

Table 2. — Analyses of variance for the rooting rate (expressed by ASRRC), the number of roots and the length of the longest root per rooted cutting, and analysis of covariance with the number of roots as covariable for the root score.

	ASRRC			No of roots			Length		Score	
Source	DF[1]	MS[2]	F[3]	DF	MS	F	MS	F	MS	F
Block	2	0.005	0.0	2	1.058	2.6	48.836	5.7*	0.040	0.8
Donor plant (D)	1	14.675	58.5**	1	0.062	0.2	0.000	0.0	0.000	0.0
Within-shoot position (P)	7	2.942	11.7**	2	5.624	13.9**	0.694	0.1	0.037	0.7
Auxin (A)	1	8.815	35.1**	1	8.293	20.5**	8.638	1.0	0.019	0.4
D x P	7	0.234	0.9	2	1.298	3.2	1.559	0.2	0.034	0.7
D x A	1	1.742	6.9*	1	0.019	0.1	3.216	0.4	0.002	0.0
P x A	7	0.290	1.2	2	0.197	0.5	2.691	0.3	0.102	2.0
D x P x A	7	0.120	0.5	2	0.754	1.9	5.065	0.6	0.105	2.1
No of roots				1					0.685	13.4**

1) Degrees of freedom.
2) Mean square.
3) Value of Fischer's statistical test with significance levels: *: $p \leq 0.05$; **: $p \leq 0.01$.

Correlations

Analysis of correlations between traits showed that the rooting rate might be slightly positively correlated with the average number of newformed roots ($r = +0.232$, $P = 0.0603$), this latter criterion being strongly negatively correlated with the average root score ($r = -0.810$, $P < 0.01$).

Analysis of variance, covariance and comparison of means

The analysis of variance summarized in *table 2* shows significant effects of the 3 experimental factors investigated ($P < 0.0001$) and of the „donor plant x auxin" interaction ($P = 0.01$) on the rooting rate, whereas the number of roots was found to be influenced by the within-shoot position and the auxin treatment ($P < 0.0001$). The length of the longest root was observed to be influenced only by the date the different blocks were set ($P = 0.0113$).

To summarize, the following results were obtained (see *Table 1* for accurate data):

1. Cuttings taken from seedlings exhibited higher rooting rates than those from stump sprouts of a 6-year-old ortet.

2. There was a decrease of rooting potential of cuttings from the top to the base of the shoot they had been collected from. This was more pronounced for the mature material as illustrated in *figure 1*. Student-Newman and Keuls' multiple range test confirmed that the terminal shoot cuttings (position 1) rooted overall in greater proportions than those located underneath, exhibiting in ad-

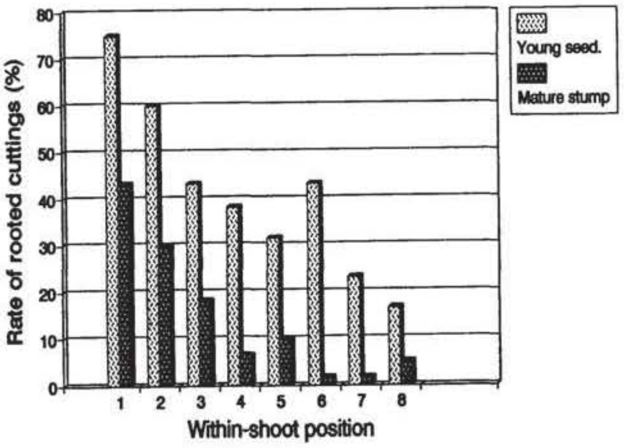

Figure 1. — Within-shoot position effect on the rooting rate for cuttings (RRC) collected from young seedlings ("Young seed.") and a mature stump of *Acacia mangium*.

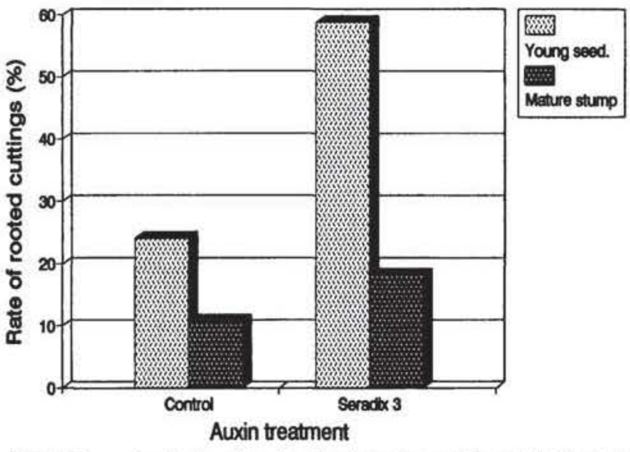

Figure 2. — Auxin treatment effect on the rooting rate for cuttings (RRC) collected from young seedlings ("Young seed.") and a mature stump of *Acacia mangium*.

dition more roots and lower root scores than cuttings taken from positions 2, 3, 4 and 6.

3. Cuttings treated with Seradix 3 were shown to root better, producing more roots than the untreated ones.

4. Seradix 3 was more effective on cuttings taken from seedlings than on those removed from stump sprouts, illustrated in *figure 2*.

Discussion

The results obtained from this experiment established that the overall capacity for adventitions root formation by *Acacia mangium* cuttings could be significantly influenced by 3 factors.

The age of the donor plant has in fact been often reported to act negatively on the ability of cuttings to form adventitious roots (BONGA, 1982; HACKETT, 1985). In *Acacia mangium*, negative maturation effects on the capacity of ortets to be vegetatively propagated through rooted cuttings seem to appear very early (DARUS, 1991). The experiment reported here demonstrated that even sprouting shoots from a mature stump close to the root system, usually considered to be the most responsive plant material (BONGA, 1982; HACKETT, 1985), showed much lower rooting rates than juvenile 6-month-old seedlings. The latter exhibited a weaker overall potential for adventitious rooting than the 56-day-old *Acacia mangium* seedlings tested by WONG (1989) in similar experimental conditions, which further demonstrates the negative influence of maturation on rooting ability, in agreement with the findings of HAINES et al. (1991). According to HACKETT (1988), the difference in terms of rooting rates established between the 6-month-old seedlings and the mature stump sprouts might be due to a deficiency in endogenous promoters or an excess of inhibitors of adventitious root induction in the mature plant material. This is supported by the fact that once rooted, the cuttings displayed similar root system characteristics, regardless of the age of the donor plant. Rooted cuttings of *Acacia mangium* seem to give rise to only a limited number of roots ranging from 2 to 3 on average, even in the case of very young seedlings (WONG, 1989). This could account for the strong correlation found between the number of roots and the root score (*Table 2*). Further investigations are needed to check possible detrimental impact of so few adventitious roots on the quality of the plantations.

Keeping in mind the growth regulator influences, it is not surprising that the cuttings from the top of the shoot, close to the terminal bud where endogenous auxin is assumed to be synthesized (CHAUSSAT and COURDUROUX, 1980; HARTMANN et al., 1990), demonstrated greater capacity for adventitious rooting than those from lower positions. This result however is not consistent with the observation on 56-day-old *Acacia mangium* seedlings where nodal cuttings corresponding to positions 3 to 5 gave the best rooting rates (WONG, 1989). In that latter case, it might be hypothesized that extreme tenderness of the terminal shoot cuttings was responsible for early mortality due to irreparable hydric stress. Another hypothesis related to this within-shoot basipetal decreasing gradient of rooting ability could be associated with anatomical and histological features. The newly formed tissues from the upper part of the shoot may be more prone to form adventitious roots than tissues from lower parts of the shoot that are more differentiated and ontogenetically older, in which the cylinder of sclerenchymatous cells reported by DARUS (1989) may constitute and obstacle to root formation and further development.

The beneficial effect of treating the base of the cuttings with "Seradix 3", an exogenous auxin, to improve the rooting rate as well as the quality of the adventitious root system is in agreement with the observations of several authors (DARUS, 1989; WONG, 1989). More interesting is the interaction between the auxin treatment and the age of the donor plant. Several hypotheses can be taken into consideration, such as better receptivity of juvenile tissues to the synthetic auxin applied, or major differences between juvenile and mature plant material in endogenous factors involved in adventitious root formation, as reviewed by HACKETT (1988).

Conclusion

The results obtained in this experiment tend to confirm that the overall ability of *Acacia mangium* to be propagated by rooted cuttings remains rather limited, especially from mature selected ortets. Furthermore, mature selected *Acacia mangium* may fail to sprout from the stumps once they have been felled. This restricted potential for adventitious root formation has to be fully taken into consideration in tree improvement and development programmes for *Acacia mangium*.

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Performance of Open-Pollinated Progenies of Blue Pine in Romania

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Summary

Genetic variation in 8 traits, including height growth and resistance to blister rust (*Cronartium ribicola* FISCH ex. RABENH.), was observed in 36 blue pine (*Pinus wallichiana* A. B. JACKS.) open-pollinated progenies. Results after 11 growing seasons were as follows: (1) Highly significant ($p<0.001$) genetic differences were observed among families for most traits; (2) The genetic components of variance accounted for 91%, 99%, 96%, 79%, 38%, 36% and 38% of the phenotypic variance for blister rust resistance, trees free of blister rust, survival, total height growth, diameter at 1/2 height, basal area at 1/2 height and stem volume, respectively; (3) The narrow-sense heritability estimates for the above traits were 0.909, 0.998, 0.960, 0.794, 0.379, 0.360 and 0.380, respectively; (4) No significant correlations were found for blister rust resistance and any growth traits; (5) The frequency distribution of blister rust resistance and height growth suggest a polygenic control; (6) Selecting the best 5, 10, 15 and 20 out of 36 families would result in genetic gain for blister rust resistance of 67%, 51%, 40% and 30%, and 18%, 14%, 11% and 8% for stem volume.

Key words: Pinus wallichiana, Cronartium ribicola, open-pollinated progenies, rust resistance, growth traits, genetic variance, heritability, genetic gain.

FDC: 165.53; 443; 181.6; 232.12; 172.8 *Cronartium ribicola;* 174.7 *Pinus wallichiana.*

Introduction

Blue pine grows throughout the Himalayan Mountains between latitudes 25 °N and 36 °N and longitudes 68 °E and 100 °E with a discontinuous pattern from eastern Afghanistan to Yunnan Province, China. It is an important component of middle and high elevation forests in this region (CRITCHFIELD and LITTLE, 1966) and is found between 1320 m and 4125 m. The best growth occurs between 1980 m and 2970 m (DOGRA, 1972).

Blue pine is a moderate to fast growing species and is one of the most important multipurpose timber species within its natural range. With its extensive range over a variety of habitats, and with its discontinuous distribution, genetic variation is a real possibility (AHSAN and KHAN, 1972).

Using artificial inoculation techniques, blue pine was found to be moderate to highly resistant to blister rust (HEIMBURGER, 1962, 1972; BINGHAM, 1972a; HOFF et al., 1980; DELATOUR and BIROT, 1982; STEPHAN, 1986; BLADA, 1987).

Blue pine is useful in interspecific hybridization programs. When crossed with other white pine species it has produced hybrids that are both resistant to blister rust (HEIMBURGER, 1958, 1962, 1972; PATTON, 1964; ZSUFFA, 1979b; BLADA, 1987, 1992) and good growth (RIGHTER and DUFFIELD, 1951; WRIGHT, 1959; ZSUFFA, 1979a; LEANDRU, 1982; KRIEBEL, 1983; BLADA, 1987, 1992; KRIEBEL and DOGRA, 1992).

The IUFRO Committee on Resistance to White Pine Blister Rust selected blue pine as its first choice for immediate attention for the following reasons: good growth and relatively high level of rust resistance; an extensive natural range and, thus, a greater probability of genetic variability for important traits such as rust resistance, growth characteristics and hardiness (BINGHAM et al., 1972). Field trials in Ohio (USA) (KRIEBEL and DOGRA, in preparation) suggest that: (1) Most of the trees from the sources of blue pine tested at this location suffered annual injury from spring frosts that resulted in multi-stemmed trees and reduced growth; (2) Growth rate of blue pine varies widely with seed source; and (3) *Pinus strobus* x *P. wallichiana* hybrids were not damaged by frost and they have a higher specific gravidity than *P. strobus*. GARRETT (1993) observed 56 half-sib families from 6 provenances in Pakistan and noted that survival was good to excellent for all sources (79% to 92%) after 9 growing seasons in southern Maine (USA) at latitude 43°30'N and longitute 70°45'W. There was no visible injury to buds or shoots that could be attributed to cold temperatures in spring or fall. Attacks by the white pine weevil (*Pissodes strobi* PECK.) that killed the terminal shoot ranged from 47% to 86% in blue pine and this level of attack severely retarded height growth. The variation within provenances suggested that more work is needed to properly evaluate the potential of this tree as a pure species or in hybrid combination with other white pines in North America.

The objective of this research in Romania was to test a limited number of open-pollinated families for rust resistance and growth traits.