Needle Characteristics of Hybrids of Some Species of Southern Pine

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Abstract

Nine needle traits were evaluated as indications of hybridity between longleaf (Pinus palustris MILL.), slash (P. elliottii var. elliottii ENGELM.), loblolly (P. taeda L.), and shortleaf (P. echinata MILL.) pines. Hybrids and representatives of each parent species were grown on a cleared forest site and on an old field. The usefulness of each trait for discriminating the hybrid from their parents was estimated on the basis of its degree of intennediacy in the hybrid and its contribution to a multivariate expression.

The utility of individual traits varied by site; fewer traits were required for effective discrimination on the infertile, cleared forest site. Over both sites, traits mast useful for discriminating longleaf X slash were: sheath length, needle length percentage of 3-needled fascicles, and rows of stomata for short leaf X slash: percentage internal resin canals, sheath length, stomata per cm and needle diameter; for slash X loblolly: percentage internal resin canals, stomata per cm, percentage 3-needled fascicles, sheath length, rows of stomata, needle diameter and stomata per cm. Serrations per cm and number of resin canals per needle were of no value for any hybrid.

Traits which most clearly exhibited intermediacy, and which may be useful in genetic or complex hybrid investigations were: for longleaf X slash — sheat length; for shortleaf X slash and shortleaf X (shortleaf X slash) — needle length, needle diameter and percentage of internal resin canals; for slash X loblolly — stomata per cm and percentage of internal resin canals; and for shortleaf X loblolly — needle length.

Key words: Southern pines, hybrid discrimination, needle traits, heterosis, additive inheritance.

Zusammenfassung

Hybriden zwischen Pinus palustris MILL., *Pinus* elliottii var. elliottii ENGELM., Pinus taeda L. und Pinus echinata MILL. wurden an Hand von Nadelmerkmalen mit den Elternbäumen verglichen. Bei den jeweiligen Hybriden waren die Länge der Nadelacheide, der Anteil dreinadeliger Kurztriebe, der Nadeldurchmesser usw. je nach Arten — Kombination geeignete Beziehungsmerkmale zu den Eltern — Arten.

Introduction

Needle characteristics are frequently used to identify hybrids of species among the conifers (KENG and LITTLE, 1961; SCHMIDTLING and SCARBROUGH, 1968). Many traits have been employed but little information concerning their usefulness is available. Ideally, discriminative traits should differ substantially between species, be inherited in an additive manner, and be unaffected by genotype X environment interaction. In this report, nine needle traits are evaluated for their utility in certain known hybrids of Pinus palustris MILL., P. elliottii var. elliottii ENGELM., P. taeda L., and P. echinata MILL.

Materials and Methods

Hybrid crosses were made on trees of the parent species growing in southern Mississippi and consist of: longleaf X slash, shortleaf X slash, chortleaf X (shortleaf X slash), full-sib families per hybrid were produced by using different individual parents. The hybrids and wind-pollinated families from the parent species were grown together on two contrasting sites. In this study, the wind-pollinated families represent the parent species (*Table* 1). Generally, wind-pollinated families were from different individual parents than those used in the hybrid crosses. Area A was set out in a three-replicate randomized block design and was planted in 1957 on a relatively infertile, well to excessively drained, cleared forest site; area B consisted of seven blocks and was planted in 1959 on an old field site having better fertility and moisture conditions. SCHMITT (1968) described the plantings in detail.

Mature needles were mllected from the lower crowns of individual trees in area A in July 1961 and in B in July 1962. Collections were made in all blocks and camposited. The average number of trees sampled per parent species or hybrid was 83, but varied from seven to 108. Six fascicles of needles were collected per tree in area A and three in area B. Fascicles were stored in vials of an FPA solution (10-formaldehyde, 5-proprionic acid, 50-alcohol, 35-water by volume). The characteristics rneasured on collected material were:

1. Number of needles per fascicle.
2. Needle length to the nearest mm.
3. Fascicle sheath length to the nearest mm on the extended sheath.
4. Needle diameter to the nearest 0.1 mm.

Characteristics measured on one needle per fascicle were:

1. Number of serrations per cm of needle length.
2. Number of stomata per cm of needle length.
3. Number of rows of stomata per needle.
4. Number of resin canals.
5. Position of resin canals.

Serrations, stomata per cm, and rows of stomata were counted on midsections using a dissecting microscope at 25X and reflected light. Free-hand cross sections were made, mounted in water under a cover slip, and examined with transmitted light at 100× to determine number and position of resin canals. Position was recorded as internal (touching the endodermis), or medial (surrounded by mesophyll), and the proportion 09 internal resin canals was calculated. Tree, family, and hybrid averages were then computed.

As a first step in preparing for multivariate analyses, trait means for individual trees of each family were observed graphically in multi-dimensional plots (NANCE, POLMER and KEITH, 1975). Outliers, which possibly were not hybrid~were removed from the data set. The procedure was then repeated with individual family means within species or hybrids to determine if observations from the two sites could be combined. Generally, data from the two plantations for a family or hybrid were not homogenous enough for cornbined statistical analysis. Therefore, separate multivariate analyses were perfonned, and any combined statistics presented are averages found after correction for scale effects where necessary.

Next, data were subjected to multivariate discriminate analyses acmrding to Program MDA-H1H2 (COOLEY and LOHNES, 1962) which gave univariate and multivariate statistics. For this the data for all families of a species or hybrid were combined. A two-group discriminant function was derived for the parent species of each hybrid. This function was used to compute the values for the discriminant axis here called the multivariate trait. These values, calculated for the two parent species and the hybrid, were plotted as frequency distributions to show the degree of discrimination. Separation between the two parent species

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Table 1. — Means of needle characteristics by species and hybrids in Area A (cleared forest) and Area B (old field)

Species or hybrid	Area	Families No.	Trees No.	Needle length cm	Sheath length mm	Needle diameter 0.1 mm	Rows of stomata No.	Stomata/cm No.	Serrations/cm No.	3-needled fascicles %	Resin canals /needle No.	Internal resin canals (%)
P. palustris	A	2	32	33.60	31.29	14.14	15.50	98.20	40.64	99.0	3.15	98.0
Hybrid	A	1	14	30.09	19.91	14.26	17.18	88.89	43.86	74.0	2.79	98.0
P. elliottii	A	3	54	19.80	12.39	13.07	18.37	94.78	42.28	19.0	2.59	91.0
P. palustris	B	2	55	37.02	30.44	16.88	19.37	94.95	34.04	98.5	3.88	94.0
Hybrid	B	4	92	28.36	21.92	16.03	20.79	92.22	34.42	95.2	4.44	95.2
P. elliottii	B	4	119	21.81	12.61	15.57	21.70	95.36	36.90	64.0	3.31	85.0
P. elliottii	A	3	54	19.80	12.39	13.07	18.37	94.78	42.28	19.0	2.59	91.0
Hybrid	A	2	30	17.65	10.70	12.07	19.28	108.18	49.24	78.5	2.42	43.0
P. taeda	A	2	54	17.32	11.16	12.60	23.60	129.84	56.04	98.0	2.22	11.0
P. elliottii	B	4	119	21.81	12.61	15.57	21.70	95.36	36.90	64.0[1]	3.31	85.0
Hybrid	B	5	103	19.67	11.23	15.05	23.11	104.40	43.21	98.0[1]	2.83	34.2
P. taeda	B[2]	1	7	17.21	12.14	14.45	24.63	121.31	45.43	100.0[1]	2.14	7.0
P. echinata	A	1	17	9.24	4.02	10.16	18.92	112.82	53.29	63.0	2.38	2.0
P. ech. × (*ech.* × *ell.*)	A	1	18	11.24	5.10	10.78	19.78	106.23	50.43	68.0	2.83	25.0
P. ech. × *P. ell.*	A	1	16	14.82	7.59	11.46	18.68	99.87	42.71	49.0	2.76	38.0
P. elliottii	A	3	54	19.80	12.39	13.07	18.37	94.78	42.28	19.0	2.59	91.0
P. echinata	B[3]	—	—	—	—	—	—	—	—	—	—	—
P. ech. × (*ech.* × *ell.*)	B	—	—	—	—	—	—	—	—	—	—	—
P. ech. × *ell.*	B	4	107	16.63	9.18	14.13	21.57	97.34	37.38	82.2	3.04	31.5
P. elliottii	B	4	119	21.81	12.61	15.57	21.70	95.36	36.90	64.0	3.31	85.0
P. taeda	A	2	54	17.32	11.16	12.60	23.60	129.84	56.04	98.0	2.22	11.0
Hybrid	A	1	18	12.81	6.34	11.3 8	20.98	120.67	47.11	95.0	2.34	2.0
P. echinata	A	1	17	9.24	4.02	10.16	18.92	112.87	53.29	63.0	2.38	2.0

[1]) Includes 4-needled fascicles.
[2]) Species data from Area A supplemented those in B.
[3]) Species data from Area A used.

Table 2. — The t-values of traits for separating parent species

Trait	t-value[1/]			
	Pinus palustris versus *P. elliottii*	*P. echinata* versus *P. elliottii*	*P. elliottii* versus *P. taeda*	*P. taeda* versus *P. echinata*
Multivariate trait	38.8	41.6	53.7	33.5
Sheath length	25.9	14.4	2.9	24.7
Needle length	23.7	18.1	6.7	16.6
3-needled fascicles	8.8	3.3	10.0	4.2
Rows of stomata	6.0	2.4	8.1	6.6
Needle diameter	4.5	13.2	6.3	10.9
Internal resin canals	4.0	25.2	29.9	2.3
Resin canals	3.2	3.7	6.0	1.8
Serrations	3.2	10.2	14.1	0.2
Stomata number	1.9	11.2	23.3	6.0

1/ t-values greater than 2.6 are significant.

was also measured by t-tests on the multivariate trait and compared with t-tests on the univariate traits. A stepwise regression procedure was used to determine the contributions of univariate traits to the multivariate trait (Draper and Smith, 1966).

To obtain a measure of additive gene action, the degree of intermediacy was estimated as the difference between a hybrid mean and that of the low-valued parent species and was expressed as a percentage of the difference between parent species. A similar calculation was used for backcross hybrids. Thus, hybrid values of 50% and backcross values of 75% or (25%) indicate exact intermediacy and additive gene action. Such values are here termed "dominance" values and are given only for traits for which the parent species were significantly different in both plantations. The means on which the univariate dominance analyses are based appear in *Table 1*. The 0.01 level of probability is used for testing significant differences.

Results

Pinus palustris × *elliottii*. — The parent species are well separated and most of the hybrids trees are intermediate as shown by the distributions of the multivariate trait *(Fig. 1)*. The greater efficiency of the multivariate trait compared with the univariate traits is illustrated by the t-values *(Table 2)*. Eight of the nine univariates had significant t-values. Sheath length was the best as it was for slash × sand *(P. clausa* (Chapm.) Vasey) pine (Saylor and Koenig, 1967).

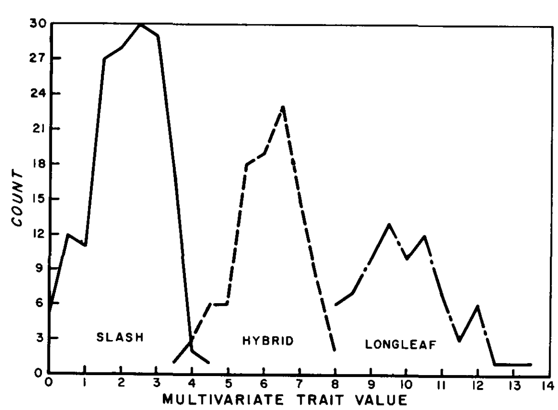

Figure 1. — Frequency distributions of the multivariate trait values for slash × longleaf. The number of trees is plotted against scaled values.

Not all of the traits contribute enough to the multivariate trait to merit use in future research. The decision on which trait to retain was made by stepwise regression. On site A, sheath length, needle length, and percentage of 3-needled fascicles, entered into regression in that order, explained 99% of the variation in the multivariate trait *(Table 3)*. On site B, sheath length, rows of stomata and needle length explain 98% of the variation. We therefore recommend using these four traits in future work. The expense and time necessary to measure the others would not be worthwhile.

Percentage of 3-needled fascicles was an important contributor to the multivariate trait only in analyses based on site A. This example of genotype × environment interaction also occurred in univariate analyses — the t-value was 13 for trees on site A and only 5 on site B — an average of 8.8 *(Table 2)*. The difference existed because slash pine has a much larger percentage of 3-needled fascicles on the old

Table 3. — The importance of traits as found by their order of acceptance in regression equations estimating multivariate traits

Cleared site (A) Trait	Cum R^2	Old field (B) Trait	Cum R^2
Pinus palustris x *P. elliottii*			
Sheath length	0.92	Sheath length	0.89
Needle length	.96	Rows of stomata	.94
3-needled fascicles	.99	Needle length	.98
P. echinata x *P. elliottii*			
Internal resin canals	.95	Internal resin canals	.83
Needle length	.98	Needle diameter	.94
Stomata number	.99	Needle length	.97
		Stomata number	.99
P. elliottii x *P. taeda*			
Internal resin canals	.95	Internal resin canals	.83
Stomata number	.98	Stomata number	.93
3-needled fascicles	.99	Needle length	.96
		Needle diameter	.97
		3-needled fascicles	.99
P. echinata x *P. taeda*			
Sheath length	.95	--	
Needle length	.97	--	
Rows of stomata	.97	--	
Needle diameter	.98	--	
Stomata number	.99	--	

field, site B *(Table 1)*. A similar effect was noted for all species comparisons involving slash pine.

Fewer traits were required to identify *P. palustris* × *elliottii* and other hybrids on the poor site than on the old field site.

With regard to intermediacy, sheath length was the only univariate trait with a significant t-value from both sites that also had a desirable dominance value (41%). The multivariate trait, however, was better with a value of 48% *(Fig. 1)*.

A check on additivity was possible because wind-pollinated material from the same three longleaf and slash parents from which four hybrids came were also available. Results for all traits were in general agreement with those where parents were identified only as to species. Using identified individual parents, the clearest case of intermediacy was found for sheath length *(Fig. 2)*. A suggestion of negative heterosis for stomata/cm in the general data was confirmed by individual family comparisons.

Pinus echinata × *elliottii*, and *P. echinata* × *(echinata* × *elliottii)* — Slash and shortleaf pines are easily distinguished as are most of the hybrid and backcross trees involving them *(Fig. 3)*. The larger t-values were: multivariate trait 42, percentage of internal resin canals 25, needle length 18.1, sheat length 14.4, and needle diameter 13.2 *(Table 2)*.

Four traits explained most of the variation in the multivariate trait *(Tables 2, 3)*. On both sites, the percentage of internal resin canals was most important. Shibata (1968) also found it useful for *P. rigi-taeda*. On the cleared site, needle length and stomata per cm were useful; discrimination on the old field site required the use of needle diameter, needle length, and stomata per cm. The other five traits were not useful.

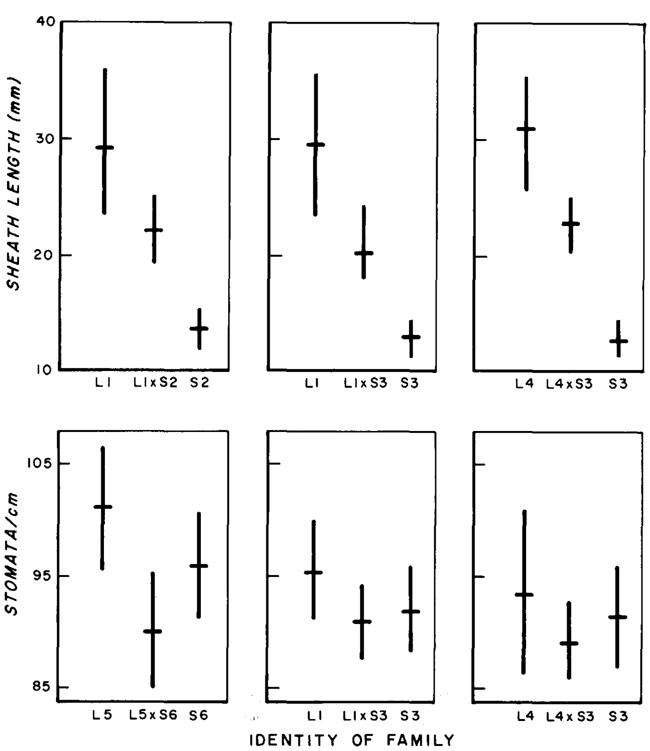

Figure 2. — Mean and standard deviation for longleaf × slash hybrids and open-pollinated families. Longleaf × wind is at left and slash × wind at right of each set. Families of parents 5 and 6 were on a different site from 1 through 4. Sheath length (top) shows additive inheritance; number of stomata (bottom) suggests a trend of heterosis.

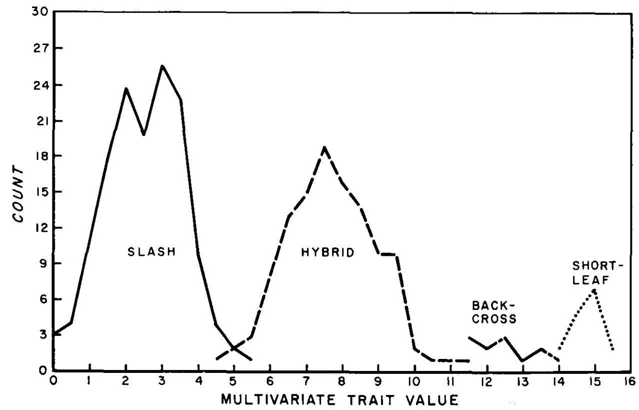

Figure 3. — Frequency distributions of the multivariate trait values for slash × shortleaf. The number of trees is plotted against scaled values.

Variation occurred in dominance for both the F_1 and backcross generations for all nine traits *(Fig. 4)*. Of those with significant t-values, the multivariate trait, needle length, needle diameter, and percentage of internal resin canals have favorable F_1 dominance values — varying from 40% to 53% and backcross values from 75% to 81%. The results are comparable to those of Nikles (1964) who used similar traits and hybrids.

Pinus elliottii × *taeda* — Complete separation of the parents with little overlap of hybrid trees is attained by the multivariate trait *(Fig. 5)*. The larger t-values were: multivariate trait 53.7, percentage of internal resin canals 29.9, and stomata/cm 23.2 *(Table 2)*. Major contributors to the multivariate trait on both sites were: percentage internal resin canals, stomata per cm and percentage of 3-needled fascicles. Needle length and needle diameter were also required on site B *(Tables 2, 3)*. Of these traits, several had desirable dominance values: multivariate trait 44%, stomata/cm 36%, percentage of internal resin canals 37%.

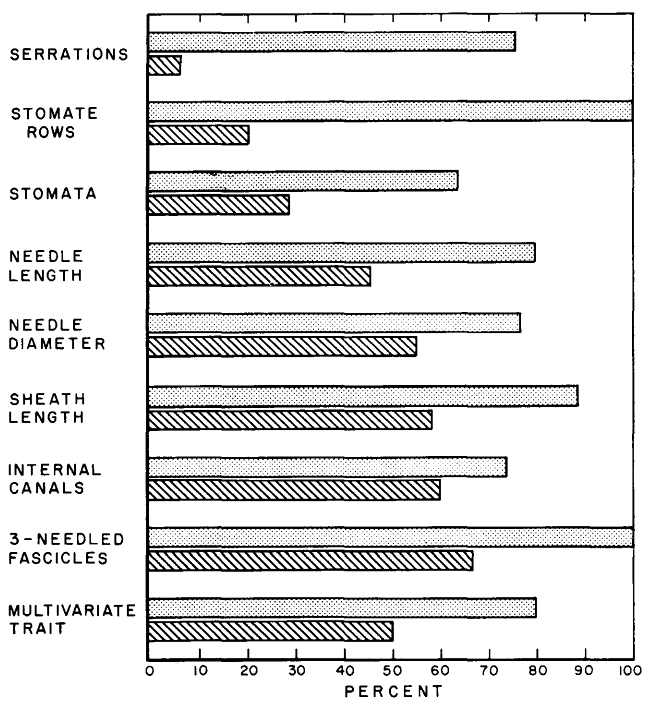

Figure 4. — Approach of *P. echinata* × *(echinata* × *elliottii)* to theoretical expectations of 75% (stippled bar) and approach of F_1 to mid-parental value of 50% (crosshatched bar). Parental values are represented by 0 and 100 (see text).

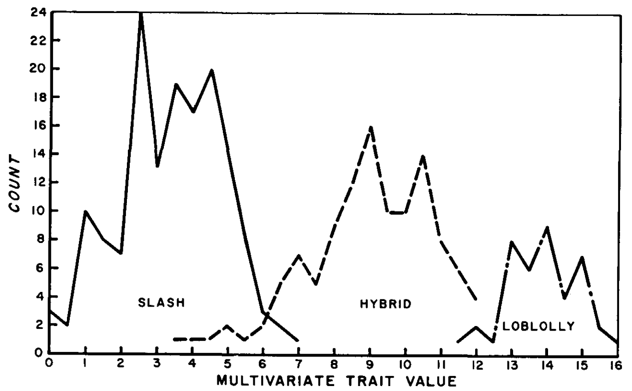

Figure 5. — Frequency distributions of the multivariate trait values for slash × loblolly. The number of trees is plotted against scaled values.

Pinus echinata × taeda — The parent species and the hybrids are distinct *(Fig. 6)*. The largest t-values were: multivariate trait 33.5, sheath length 24.7, and needle length 16.6 *(Table 2)*. Traits most useful for estimating the multivariate trait were: sheath length, needle length, rows of stomata, needle diameter, and stomata/cm *(Tables 2, 3)*. Dominance values were 41% for the multivariate trait and 44% for needle length.

Discussion

We agree with MERGEN (1958) that stomata/cm is useful for identifying slash × loblolly hybrids, and that stomata/cm and serrations/cm are not discriminatory for longleaf × slash. Similarly, for loblolly × shortleaf hybrids, HICKS (1973) and FLORENCE (1973) rated needle length, sheath length, and needles/fascicle as the best discriminative traits for univariate and hybrid index analyses. FLORENCE, while also employing multivariate analyses, did not identify the contributions of individual traits. For multivariate analysis, we would use needle length and sheath length as did FLORENCE, plus rows of stomata, needle diameter, and stomata number. For multivariate discrimination, WELLS, NANCE and THIELGES (1977) separated shortleaf and loblolly chiefly on needle length, stomata/cm, and number of resin canals. Whether dicrepancies in apparent usefulness of some of the traits are caused by differences in provenance, sample sizes, or measurement methods is not known.

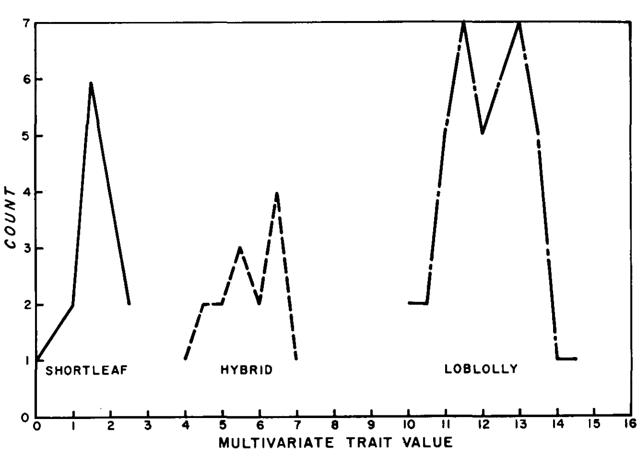

Figure 6. — Frequency distributions of the multivariate trait values for shortleaf × loblolly. The number of trees is plotted against scaled values.

Some traits vary in usefulness according to site. For example, SMOUSE (1971) has correlated needle length with "energy" available. MERGEN, STAIRS and SNYDER (1965) noted that shortleaf × loblolly could be distinguished better from parent species on a poor site than on a good site. We found this was also true of other hybrids. It will therefore be necessary to conduct genetic studies of such traits within a site or apply adjustments when experimenting across sites.

Since information on the direction and degree of dominance is reliable only if differences between parents are significant, disagreements and agreements among researchers as to dominance should be re-examined. We disagree with the findings of MERGEN *et al.* (1965) that stomata/cm, numbers of stomata rows, and numbers of resin canals show heterosis in loblolly × shortleaf hybrids. Such discrepancies are not unexpected considering our low t-values for these traits. Even in the case of agreement, such as heterotic serrations/cm, there is still low confidence in the results because of a t-value near 0. However, their hybrid needle length was nearly equal to that of loblolly while ours was intermediate. In this case our t-value was high, so possibly this is a genotype × year interaction or other effect.

Our research indicates that for any one hybrid only a proportion of needle traits are efficient indicators of hybridity. Clearly, future efforts to discriminate other hybrids should be preceded by preliminary investigations of representative materials to establish the utility of particular characteristics.

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