

Identification of European and Japanese Larch and Their Interspecific Hybrid with Morphological Markers: Application to Young Seedlings

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Abstract

Open-pollinated hybridisation seed orchards of European and Japanese larches produce mixed *progenies* combining a highly variable proportion of hybrids along with pure parental species. For several reasons, it is desirable to identify and to sort out hybrids from pure species at the seedling stage. Taxa identification of 1-2 yr-old seedlings was attempted using non-destructive assessment of several traits, including morphology, phenology, growth and architecture parameters. Two sets of progenies originating from 10 open-pollinated hybridisation seed orchards were used, relying in a first step on taxa identification of individual seedlings with diagnostic molecular markers. Based on 21 traits assessed, some clear trends in pure species and hybrid features were apparent but due to the large and overlapping ranges of taxa characteristics, no single parameter allowed unambiguous identification of taxa. Combination of traits through linear discriminant analysis made possible correct classification of 90.2% to 98.6% of individuals depending on the orchard although there were a few problematic orchards. Two traits appeared particularly pertinent for discriminating young plants taxa, namely 1st-yr leaf retention (marcescence) and the bark colour of 2nd-year shoot increments. Results were corroborated using progenies from several orchards and over two experimental periods.

Key words: *Larix*, taxonomy, hybrid, seedling, morphology, phenology, growth, linear discriminant analysis, seed orchard.

Introduction

Among species of the *Larix* genus, two are of particular importance for Western European forestry, namely European larch (*Larix decidua* coded 'EL') and Japanese larch (*L. kaempferi* (Lamb.) Carr, coded 'JL'). Their interspecific hybrid (coded 'HL') proved also to be of high value for lowlands reforestation and since its first observation in Scotland at the beginning of the 20th century (HENRY and FLOOD, 1919), hybrid larch has been the object of intensive breeding work across Europe.

From the late 1940s on, over 30 hybridisation seed orchards have been established across Europe to mass-produce improved hybrid larch (DESTÉUCQ, 2003). Their genetic composition, design and combination types are various but nearly all of them rely on open-pollination.

Because of incomplete overlapping of the flowering periods of European and Japanese larch and often unfavourable climatic conditions during anthesis and pollination, seed production is erratic, both quantitatively and qualitatively. Besides a low percentage of filled seed and therefore low germination, the proportion of hybrids is generally low and also highly fluctuating between years. This has been revealed by a few studies using biochemical markers First (BERGMANN and RUETZ, 1987; BRAUN, 1990; HACKER and BERGMANN, 1991; ENNOS and QIAN, 1994) and more recently molecular markers (ACHERE, 1999; SCHEEPERS et al., 2000; ACHERE et al., 2004).

As a result, reforestation with so-called 'hybrid' larch has been done so far in an uncontrolled way, with varying mixtures of hybrid plants and either European or Japanese larches or both. The possibility to individually identify taxa and from a practical point of view, to sort out hybrids out of nursery benches prior to plantation, is obviously attractive for experimental purposes but also for commercial plantations where product homogeneity is sought for.

Although reliable, diagnostic molecular markers developed so far are too expensive to be used for routine sorting whereas morphological markers would be a *priori* better suited. However, sorting based on morphological markers is rather complex in this context for at least four reasons: firstly because of the young age of the material to be identified (1-3 yrs old seedlings) whereas the botanical description and classification of the *Larix* species rely mostly on mature tree characteristics (PATSCHE, 1913; OSTENFELD and LARSEN, 1930); secondly, because European and Japanese larches appear closely related both taxonomically and genetically (SEMERIKOV et al., 2003; WEI and WANG, 2003); thirdly, because seedlings to be identified have closely related pedigrees (pure species and hybrid siblings share common parents); and fourthly, because the parental clones used in the various seed orchards may cover a wide

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