Dedicated to the memory of John Rouse - a very special colleague and friend, who will be much missed by all who love rhododendrons. - Elizabeth G. Heij, March 2002.

Vireya rhododendron hybrids: an adventure in variety

Elizabeth G. Williams & John L. Rouse

from Rhododendrons 1986-7 with Magnolias and Camellias

In the genus *Rhododendron* some 270 species form a distinct section, Vireya, which is distributed through the predominantly tropical regions of Malaysia, Indonesia, the Philippines, Borneo and Papua New Guinea. These species are found mainly between latitudes 10°N and 10°S at altitudes ranging from sea level to about 13,000 feet, with a few occurring as far north as Taiwan, and in the south one species, Rhododendron lochiae, native to the coastal mountains of north Queensland in Australia. In tall forests Vireyas may be found as epiphytic shrubs on larger trees, although the same species also occur on the ground in cleared areas. At higher altitudes they occur in profusion on the ground, forming a shrubby ericaceous forest, and in extreme montane conditions may be low bushes, mats or alpine bog plants. The range of altitude-related climate zones to which Vireyas are adapted has been graphically described by Dr. R.M. Withers (1983) in the yearbook for 1983/4. Here it will suffice to point out that while certain species of this group are adapted to hot, humid tropical jungles, others are adapted to the periodic freezing temperatures of the high mountains or to the more temperate climates of the mid-altitude range.

Vireyas are distinguished botanically by the two long tails on their seeds. Other distinguishing features are the indumentum, a covering of microscopic surface hairs, which is composed of small scales rather than the more typical hairs found in many other rhododendrons, and a tendency of many species towards a sparse or leggy growth habit.

Although Vireyas show a range of attractive foliage types from narrow and heath-like (*R.stenophyllum*) to broad-oval (*R.orbiculatum*), it is their flowers, with their particular purity and brilliance of colour, which are the principal feature. They range in shape from tubular (*R.loranthiflorum*), to bell-shaped (*R.aequabile*), or trumpet-shaped (*R.lochiae*), and in size from less than a third of an inch (*R.anagalliflorum*), to over 4 inches (*R.leucogigas*) in length. Flower colours range from white through yellow and orange to brilliant reds and pinks. There are no true blues but two species, *R.stevensianum* and *R.atropurpureum* have magenta flowers.

Vireya species may have a defined flowering season, particularly from the high altitude zones, but species from the lowland areas with little seasonal climatic variation may flower intermittently throughout the year. Thus, in a temperate climate such as that of Melbourne, with careful planning it is possible to have at least one Vireya in flower at any time of the year. The bright golden flowers of *R.laetum*, for example, can provide a welcome splash of sunshine in our winter gardens.

An important feature of Vireyas is their promiscuity. In contrast to many other groups of related plant species in which hybridization does not occur or is achieved only rarely and with great difficulty, many Vireya species can be cross-bred with relative ease to produce a whole new array of flower forms and subtle colour combinations. In fact, as a general rule of thumb, and with the exception of a few species in subsection Pseudovireya, any Vireya species is likely to be cross-fertile with almost any other, provided the lengths of their styles are reasonably similar. That is, pollen from a short-styled species may be unable to reach the ovary of a species with a very much longer style. In nature the species are prevented from hybridizing by their restriction to different geographical regions, different climate zones, and presumably to some extent by the fidelity and restricted foraging of their natural pollinators. For example, white, perfumed flowers are believed to be predominantly pollinated by moths, while yellow and orange-flowered species are favoured by butterflies. Others, particularly those with red flowers may be more attractive to birds, and some have floral markings visible only in the ultra violet spectrum to which the eyes of bees are known to be sensitive. Natural hybrids do occur, however, particularly where the regions occupied by distinct species are adjacent or overlap. An example grown from seed collected from *R.saxifragoides* in Papua New Guinea by P. Kores is believed to have resulted from pollination by *R.womersleyi*. Both species are found in the same altitude zone between 10,800 and 13,000 feet.

Both amateur growers and professional horticulturists have been hybridizing Vireya rhododendrons since they first came to the attention of the civilized world some 100 years ago. Besides a sense of adventure in making new crosses on the off-chance of breeding a 'winner', there are a number of specific reasons for attempting particular crosses: for example, the introduction of cold hardiness, heat tolerance or disease resistance, improvement of flowers, flower to leaf size ratio, foliage type, propagation qualities or plant growth form, and alteration or extension of the flowering season. All these objectives are primarily horticultural and commercial, but our own major reason for an extensive crossing programme involving the Vireyas has been to investigate compatibility relationships and major breeding barriers within the entire genus *Rhododendron*. A number of interesting new hybrids arising from the programme have provided a welcome bonus.

At this point we need to pause and define types of hybrid. A first generation hybrid resulting from cross-pollination of two distinct pure species is known as an F₁ hybrid. It is usually more or less intermediate between the parent species in a majority of characteristics, although particular features of one species may dominate. Hybrids involving *R.commonae* or *R.lochiae*, for example, tend to show the red flower colour of these two species even when the other parent may have a distinctly different flower colour such as white or yellow. Being sometimes partially or highly sterile, F₁ hybrids may be of limited

use for further breeding, although they may be propagated as horticultural varieties by means of cuttings. Sterile flowers often have the advantage of longer vase life, and dead flowers do not have to be removed from bushes by hand to ensure annual flowering.

When a fertile F_1 hybrid is self-pollinated the progeny are referred to as F_2 hybrids. Among these plants genetic segregation can produce an array of characteristics ranging from one parental extreme to the other, and also many new recombinations of characters from both. This is where the most surprising and unusual variations turn up. Further generations produced by selfing F_2 plants, F_3 and F_4 etc., may also yield novel recombinations of the original species characters, but it is possible to move gradually towards a desired combination by consistent selection of appropriate individual plants in each generation - a slow business for rhododendrons when the time from seed to flowering and selection may be at least four to five years.

When a fertile F_1 hybrid is crossed to one of its parent species this is known as a first generation backcross or BC₁, for example (*R.macgregoriae* x *R.lochiae*) $F_1 \times R.macgregoriae$. The BC₁ progeny will naturally tend to show a predominance of characters from the recurrent parent. Successive backcrossing, BC₂ and BC₃ etc., is used when it is required to introduce one particular character such as disease resistance or a new flower colour into the recurrent parent. Again plants are selected for the desired combination of characters in each generation. When a fertile F_1 hybrid is crossed to a third species, the progeny are tri-specific hybrids, that is, they carry a complement of hereditary characters from the three species, for example (*R.phaeopeplum* $\times R.lochiae$) $F_1 \times R.zoelleri$.

When setting out to produce a hybrid it is important to select healthy, vigorous parent plants with desirable features. Flowers of the plant which is to act as seed parent are then emasculated by gently removing the anthers, which contain the pollen, at or just before the time of flower opening (i.e. before the pollen is shed). This helps to prevent accidental self-pollination while handling

the flowers. The emasculated flowers are then enclosed in muslin or a paper bag to prevent contamination with unwanted pollen by visiting insects. When the stigma in the centre of the flower becomes shiny and sticky with a fluid exudate it is receptive and ready to receive pollen. The exudate ensures that pollen adheres to the stigma, and also provides the correct medium in which it can germinate to produce the pollen tube that carries the sperms down to the ovary. For an excellent and detailed description of reproductive processes in rhododendrons the reader is referred to an article by Dr. Barbara F. Palser which appeared recently in *The Rhododendron* (1985). Once the stigma is receptive, living pollen from the second parent species is spread liberally from whole anthers on to the stigma surface. Flowers should be labelled with the details of the cross and date of pollination - jewellers tags are sufficient. The flowers are then returned to their protective bags until about two weeks after pollination when the danger of contamination by stray pollen is over. It is most important that no self-pollen from the seed parent reaches the stigma, either before or after cross-pollination. One of the most depressing outcomes of an attempted hybridization is to find, when the putative hybrids flower, that one has been carefully nurturing for years a very ordinary group of selfed offspring.

When attempting crosses between species which flower at different times, special procedures may be required. Stigmas will normally remain receptive and ovules viable for several days to a week after the first signs of receptivity. It may be possible to catch the last receptive stigmas of one species with the first mature pollen of a second species (or vice versa) but if the flowering seasons do not overlap at all then pollen storage will be required. Pollen normally survives only a few days at ambient temperatures, but when dehydrated and frozen can be stored for pollination at any season of the year. After selecting recently opened flowers, pollen is most conveniently collected in the anthers, which are wrapped in a small square of thin tissue paper and placed in a small, paper seed envelope. Envelopes are kept in a closed jar over a drying agent such as anhydrous calcium chloride or blue indicator silica gel, and are held in a refrigerator at 4°C for two days, before transferring the

sealed jar to a freezer at -20°C. Pollen stored in this way will generally keep its viability for one to several years. When pollen is taken from the freezer for use, it should be allowed to come to room temperature before opening the packet. This will prevent moisture from condensing on the anthers and making the pollen difficult to handle. Care must be taken not to thaw and re-freeze unused pollen when removing packets from storage, since multiple freezing and thawing cycles can be lethal. It can be unwise to assume that pollen from an untested parent plant is viable. As already mentioned, many interspecific hybrids are partially or completely sterile, and even some individual plants of pure species may produce little or no living pollen. A common clone of *R.orbiculatum*, for example, although capable of producing seed, is completely male sterile and thus might be cross-pollinated without emasculation. To determine the pollen fertility of an untested plant is a simple matter with laboratory facilities and a microscope. If these are not available, a reliable, although slow method is to use the pollen on emasculated flowers of another clone of the same species and wait to see if capsules are set. (Selfpollinating may not be a reliable method, as a few rhododendron species show reduced or no seed set after selfing). A more rapid but less predictable method is to sprinkle pollen on the surface of a drop of 10 to 15 per cent sucrose (sugar) solution and incubate in a closed container, to prevent evaporation, for about four to six hours. Examination of the droplet with a microscope or powerful hand lens in strong light against a dark background, should reveal pollen tubes growing from the grains if they are viable. Not all species pollen grains, however, are equally willing to germinate under these conditions. Addition of small amounts of boron and calcium salts to the medium may improve germination (Lin, Uwate and Stallman, 1977).

After cross pollination, ripening capsules should be removed from the plant just as the valves begin to open at the top. Each capsule is then sliced open longitudinally along its fracture lines and allowed to complete drying for 24 hours at room temperature. The seed is then gently brushed free from the capsule, and if it is not to be planted immediately should be stored in a manner similar to that used for pollen. The seed of all Vireyas, both species and hybrids, has a much shorter lifetime than that of other rhododendrons. For many Vireya species, seed survives only about three weeks to two months in normal shelf storage conditions. This short shelf life necessitates the special care of freeze storage.

Even in normal compatible pollinations between plants of the same species, not all of the ovules develop into mature viable seeds. In mature capsules unpollinated or aborted ovules form a powdery chaff from which viable seeds can be distinguished by their greater size and central swelling representing the embryo. Capsules produced by foreign cross-pollination often contain a lower than normal number of viable seeds. Chaff from such capsules should be carefully sorted to ensure that no good seed is lost. It seems that a critical number of pollinated and developing ovules are required within the capsule to prevent it from abscising before maturity. The developing ovules are presumed to secrete a hormone which keeps the capsule stalk healthy and functional. Abscission of capsules with a lower than critical number of developing seeds is frequent in foreign cross-pollinations. In some such crosses small differences in plant health or environmental conditions appear to be quite important in determining whether capsules with hybrid seeds will be retained on the plant to maturity.

For germination, Vireya seed requires temperatures in the range of 15 to 30°C, a relative humidity above 90 per cent, sufficient free water for the seeds to imbibe and adequate light (about 4,000 lux). The germination medium should retain water and contain no inhibitors. Finely sieved moss peat is ideal, and partially decayed pine bark is also satisfactory. To prevent fungal infections and algal growth, pod chaff should be removed from the viable seeds; containers and potting media should be sterilized and all water either boiled or distilled. Some Vireyas, and especially slower growing hybrid seedlings, can be extremely sensitive to chemical fungicides, so their use should be minimized. Fungicides are no substitute for cleanliness, but if necessary Thiotox is a suitable preparation.

Cotyledons have normally emerged by about three or four (sometimes five to six) weeks after sowing. At this stage the seedlings should be ventilated to reduce the relative humidity and risk of fungal infection. Suitable conditions for early growth are: temperatures of 20 to 30°C by day and 10 to 20°C by night, relative humidity of about 80 to 90 per cent, daylength of 15 to 18 hours and illumination in the range of 2,000 to 10,000 lux. Misting may be beneficial if given for 10 seconds once or twice a day in dry or hot weather, but the seedling leaves should be dry by nightfall to help prevent fungal growth. Air should be gently circulated, with some fresh throughput, and seedlings should be watered once each week with a 10 per cent normal strength liquid fertilizer.

Naturally, after making an interspecific pollination it would be nice to know whether the emerging seedlings are indeed hybrids. If both parents were Vireyas, confirmation of hybridity may take some time, and may even have to wait until the plants flower. All Vireya species and hybrids look much alike at the seedling stage, and all have rather similar flat scales on the surface of the first true leaves. For some hybrids an intermediate leaf type may become more obvious with time, but for most a comparison of the flowers with those of the parent species may be needed. Low seedling vigour, and later on partial or full pollen sterility are other frequent indicators of hybridity. For the occasional rare hybrids we have obtained between Vireyas and azaleas we have been able to identify hybrids at the seedling stage as having hybrid indumentum hair types: 'glandular' hairs with a knobbed tip, rather than Vireya scales or azalea simple hairs. Such immediate recognition is unusual, however, and patience is a necessary breeding skill.

Seedlings can be pricked out into pots when they reach about 1/3 inch in height. This is normally at about 20 to 30 weeks after sowing, but may be longer for hybrids with low vigour. The potting mix should be light with good drainage and aeration. It should retain water available to the plant, have a slightly acid pH in the range of 4 to 6, and the soluble salts level should not be too high. The requirements for acidic conditions is general for rhododendrons throughout their growth. Ideally, pots should be misted once per day in the

morning, and the light level should be relatively high (about 5,000 to 30,000 lux). Potting on requires a similar mix to that used for pricking out, but with slightly higher nutrient levels. It is worth noting that calcium is important to rhododendron health, but it should be applied as gypsum (calcium sulphate), which does not raise the pH, as does lime.

It may be tempting to discard weak, unattractive plants, but it makes better biological sense to carry all potentially hybrid seedlings forward if possible until they are proved to be non-hybrid, or both sterile and horticulturally undesirable. Fertile hybrids which are not themselves of any horticultural merit, are still potentially valuable for transferring genetic traits from one parent species to the other by backcrossing, or for further exploratory crosses to other species or hybrids. If space permits, it may pay to take several cuttings from putative hybrid plants even before hybridity is confirmed. This will lessen the risk of loss by death of a single plant, and give more material for breeding or propagation if hybridity is verified. Most Vireyas grow well from cuttings and are often easier to root than many other rhododendrons and azaleas. The rooting mix should be similar to that used for pricking out, but without fertilizer. Cuttings should be treated with a rooting hormone such as 0.2 per cent IBA powder in talc or 1 per cent IBA liquid dip, and should if possible be given bottom heat (20 to 25°C). Their tops should be kept cool with occasional misting, and out of direct sunlight. Once rooted, the cuttings are best hardened off for about two weeks in a cool low-light area with daily misting but without bottom heat or fertilizer. They can then be brought into full glasshouse light or diffuse sunlight and given dilute liquid fertilizer weekly. Vireya cuttings are generally well-rooted in about 8 to 10 weeks, although some hybrids may take longer.

All of the above discussion has assumed that hybrid seeds will be produced and will germinate. Unfortunately this does not always happen. There are a number of things which can go wrong, and 'Murphy's Law' applies to rhododendron breeding just as it does to our other endeavours. Obvious reasons for failure of crosses to set seed are: the seed parent is in poor health, under stress or sterile;
the flowers on the seed parent are too old and past their period of receptivity;

the pollen is not viable, perhaps too old, or derived from a sterile plant;
pollen from a short-styled species has been applied to the stigma of a very much longer-styled species, and pollen tubes cannot grow down to reach the ovules.

Less obvious reasons relate to the phenomenon of interspecific incompatibility (Knox, Williams and Dumas, 1986), which has also been called 'incongruity' by some breeders. These barriers to hybridization can operate before or after fertilization. Growth of pollen tubes through the pistil of the flowers is controlled by what we might term a 'molecular conversion'. The pistil secretes signal molecules to which the pollen tube can respond by growing normally, stopping or changing direction. The pollen tube must have receptor sites which 'recognize' these highly specific signal substances. As the various species have gradually diverged from each other in evolution, there have also been mutational changes in the substances involved in controlling pollen tube growth in the pistil. Thus, if pollen from one species is put on to the pistil of a different species there is a very good chance that the pollen tubes will not be able to 'read' correctly the foreign molecular signals of the pistil. Pollen tubes may grow abnormally, twist, burst, fail to reach the ovules or fail to release sperms at the right time. The process of fertilization can fail at any one of a number of sites in the pistil, from the stigma surface to the embryo sac within the ovule, depending on the pair of species which have been crossed (Williams, Kaul, Rouse and Palser, 1986 and Williams, Knox and Rouse, 1982). In addition to this type of incompatibility which prevents fertilization from occurring, a hybrid embryo may abort after fertilization if the parent genetic contributions cannot function compatibly together to regulate normal development.

For most crosses with section Vireya the species are sufficiently closely related that incongruity barriers are not a problem. For some crosses, however, such as those involving subsection Euvireya with subsection Pseudovireya, seed set is frequently prevented by natural barriers of this type. As yet we have no way to overcome these biological constraints for rhododendrons, although in other plant families there have been occasional successful manipulations to produce hybrid seed by tricks such as heating or amputating the style, 'disguising' incompatible pollen by mixing it with killed compatible pollen, injecting pollen directly into the ovary, or rescuing hybrid embryos in tissue culture.

At present, for rhododendrons, it is best to concentrate hybridization efforts on groups of closely related species for which interspecific pollen-pistil incompatibility and post-fertilization seed abortion do not occur. Among the many cross-compatible species in section Vireya numerous crosses have not yet been attempted, and even where hybrids have been obtained by other workers, the choice of different individual parent plants is likely to yield unique and different results. Even among the hybrid sibling progeny of a single cross-pollination there may be considerable variation in horticultural potential. Hybridizing Vireyas is a continuing adventure. No single cross exhausts the potential of a particular pair of species. The progeny of one pollination might be interesting, but perhaps the next pollination of the same two species could produce a 'winner'.

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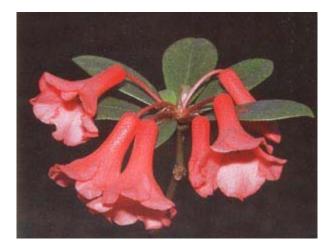
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pollen tubes in embryo sacs of *Rhododendron* following interspecific pollinations. *Australian Journal of Botany*, Vol.34 (4), pp.413-423. Williams, E.G., Knox, R.B. and Rouse, J.L., 1982. Pollination subsystems distinguished by pollen tube arrest after incompatible interspecific crosses in *Rhododendron* (Ericaceae). *Journal of Cell Science*, Vol.53, pp.255-277. Withers, R.M. 1983. The natural environment for Vireya rhododendrons. *Rhododendrons 1983/4 with Magnolias and Camellias.* Royal Horticultural Society, pp.20-24.



This natural *R.saxifragoides* alpine hybrid arose as a rogue seedling in a batch of seed of *saxifragoides* collected in the wild by Mr Paul Kores in 1976. While the other seedlings appeared to be true *saxifragoides* with low vigour, and are as yet unflowered, this hybrid developed rapidly with upright growth and is floriferous. Its leaves and flowers suggest that the male parent is *R.womersleyi* or a *R.womersleyi* hybrid and owing to its natural alpine habitat, we can expect it to be relatively cold hardy.

Seedlings obtained by selfing this hybrid are not yet large enough to flower but their vegetative growth is similar to that of their parent. Crosses (none of which have flowered yet), have been made with *R.lochiae, R.laetum, R.konori* and *R.luraluense* with the expectation of introducing cold hardiness into the offspring.



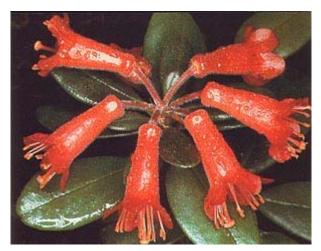
Rhododendron lochiae is the only known native Australian rhododendron. It grows in the coastal mountain tops of Queensland from Bellenden Kerr northwards. The photograph shows a specimen collected on Thornton Peak.



Rhododendron 'Australia II' is the hybrid ((phaeopeplum x lochiae) x zoelleri) x
"Gardenia aff". The female parent was grown from seed obtained from
Strybing Arboretum in 1972. The pollination with four-year-old pollen on *R*."Gardenia aff" was made in December 1978 and the seed was collected six
months later. 'Australia II' first flowered in November 1983, and was entered in
the competitive display at the Annual Spring Rhododendron Show at Olinda,
where it received the Certificate of Merit and was awarded Best Truss of a
Rhododendron Bloom in Show. A number of sister seedlings have flowered
recently. They display a wide range of flamboyant variability.



Rhododendron 'Liberty Bar' was raised from seed supplied by Mr D. Stanton who made the cross *R.aurigeranum* x *R.lochiae*. As is usually the case with F₁ hybrids when one parent is *R.lochiae*, the flowers are a bright red. This particular hybrid is most floriferous, with attractive foliage, and is well worth growing in Australian suburban gardens.



Rhododendron retusum is a terrestrial shrub from Sumatra and Java. It has dark red flowers, and because of the type of scales on the under-surface of the leaves, it is placed in subsection Pseudovireya. Species in this group are not of much horticultural interest and are seldom used in hybridization because their flowers, though attractive, are rather too small to appeal to the gardening public.



(*R.aurigeranum x lochiae*) *x retusum.* This cross was made in November 1980 and the seeds were collected and sown four months later. The aim was to study sexual compatibility within section Vireya as part of a comprehensive investigation within the genus. Here, since *aurigeranum* and *lochiae* are in subsection Euvireya, we are looking at a cross involving subsections Euvireya and Pseudovireya, two subsections which contain very different species. Other crosses of this type and reciprocal crosses, e.g. *R.retusum* x *R.javanicum*, have also resulted in hybrids, while some, e.g. *R.macgregoriae* x *R.quadrasianum* var.*rosmarinifolium*, have failed to produce viable seedlings.



Rhododendron 'Wattle Bird'. The pollination *R.laetum x R.aurigeranum* was made by J.L. Rouse in 1973 using a fine, pure yellow form of *R.aurigeranum* with many flowers in the truss, and a vigorous, large, yellow-flowered form of

R.laetum, with the aim of producing the best, brilliant yellow-flowered *Rhododendron* hybrid. A selected seedling was named and registered in

1984. 'Wattle Bird' is rather straggly, and further improvement could be expected by crossing it with *R.retivenium* which has deeper yellow flowers and shorter internodal lengths.



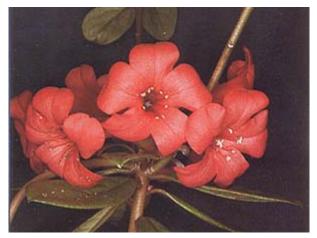
R.konori x *R.laetum.* This pollination was made in 1973 using the Irian Jaya form of *R.konori*, which has large, white, perfumed flowers occasionally with a red spot at the junction of the corolla lobes. It grows well in Melbourne, either in a container or garden bed, provided it is kept free of frost. Large plants can be expected to flower each year, with fifty or more trusses each containing three to six flowers. Unfortunately, the blooms do not usually last for long as flowering occurs in February, our hottest month. The pollination was made with the aim of observing the F1 hybrids resulting from crossing two magnificent, but very different, species. So far, seedlings which have flowered are similar and it is doubtful if any are an improvement on either parent.



Rhododendron commonae is a terrestrial shrub from New Guinea where it occurs at an altitude of 10,000 to 13,000 feet. As such, we can expect it to be more cold hardy than Vireyas from lower altitudes, a characteristic which may be transmitted to hybrid offspring when it is used as a parent. Compared to the other alpine Vireya, *R.saxifragoides*, which is difficult to grow and flower in our Melbourne climate, *R.commonae* is easily cultivated here, flowers frequently and is tolerant of our hot dry summers.



Rhododendron stenophyllum is found epiphytically or terrestrially on Mount Kinabalu in Sabah at about 6,500 feet. The orange or range-red flowers are mostly in twos or threes and when they first open the anthers are grouped together centrally at the front of the corolla, thus hiding the pistil. This species, as with most of the Vireyas, is easily propagated from seed or cuttings, but once a plant is taller than 1 1/2 feet it usually succumbs to root rot. Grafting on to a vigorous hybrid Vireya rootstock may alleviate this problem. The leaves are long and very narrow with a ratio of length:width of 25:1. When this species is used in hybridization the shape of the leaves of the small seedlings can be used to confirm true hybridity at an early stage. The photograph shows *R.stenophyllum* growing in a glasshouse at the Royal Botanic Garden, Edinburgh.



(Rhododendron konori x R.laetum) x R.commonae

and



Rhododendron stenophyllum x R.commonae

Both these crosses were made in late 1980 with the aim of producing Vireya hybrids with horticultural value and increased cold tolerance from *R.commonae*. Both hybrids have attractive foliage and flowers, but as yet their cold hardiness has not been assessed.

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