# Blackcurrant reversion disease - tracking down the causal agent

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Of about 14 distinct virus or virus-like diseases that have been recognised in *Ribes*, blackcurrant reversion is unquestionably the most important for blackcurrant crops world-wide; it also affects redcurrant but apparently not gooseberry[1]. The disease is particularly widespread in blackcurrant (*Ribes nigrum* L.) in Eastern and Central Europe and Russia, in some parts of the UK and Scandinavia[1] and, in recent years, it has become a serious problem in New Zealand (A.T. Jones, unpublished information). A form of the disease (R) present in countries of the former Soviet Union, Eastern and Central Europe, and Finland[1] is much more severe in plants than the common European (E) form but, with each of these forms, the progression of the disease in plants and crops is similar. In nature, the causal agent of the disease is transmitted between plants by the blackcurrant gall mite, *Cecidophyopsis ribis*, but not through seed. Experimentally, it can be transmitted between infectible *Ribes* plants by grafting[1].

The disease was first described in the Netherlands in 1904 by Ritzema Bos, but undoubtedly occurred before this, and presently is reported from all countries where blackcurrant is grown commercially, with the exception of the Americas. As its name suggests, the disease reflects the change in plant habit, mostly in the leaf appearance, that is suggestive of 'reversion' to a primitive wild plant type[1]. Compared to leaves of healthy plants, those of reverted plants are narrower, show a decreased number of main veins, have larger but fewer marginal serrations, and have a basal sinus that is less lobed (Fig. 1). These symptoms are usually more pronounced in infections with the R than with the E form of the disease but symptoms vary in intensity and severity between blackcurrant cultivars[4]. Another, more consistent, symptom of the disease, seen only in newly emerging flower buds, is a brighter pigmentation of the buds compared to those of healthy plants, due to a loss of the downy hairs on buds (Fig. 2). On plants affected by the R form of the disease, flower buds in addition develop strong malformations, including the absence of stamens, elongation of the style and an increase in the number of petals, and pigmentation is intensified further (Fig. 3). Affected flower buds in each form of the disease are usually sterile causing a severe loss in fruit productivity. Leaf and flower symptoms in redcurrant are less noticeable than those in blackcurrant. Much less consistent in appearance in plants affected by either form of the disease is the development in spring of foliar chlorotic line patterns and/or ringspots, as the expression of this symptom is dependent on genotype/environment interactions that are not well studied (Fig. 4). The leaf markings often disappear as the leaves age and are usually not evident on growth made during the summer. Such line-pattern symptoms are not diagnos-

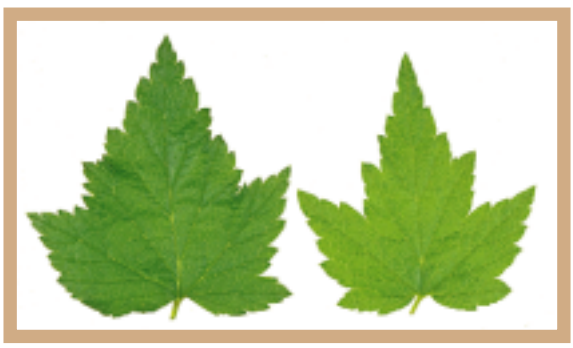

Figure 1 Leaf symptoms of reversion disease in Ben Nevis blackcurrant (right). Healthy leaf (left).

Figure 2 Brighter pigmentation of the flower buds of a blackcurrant plant affected with the R form of reversion disease (left) compared to those of a healthy plant (right).

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Figure 3 Increase in the number of petals, and intensification of pigmentation of flower parts that are characteristic of the R form of reversion. Note the erratic distribution of the symptoms in different nodes.

tic of reversion because similar symptoms can be induced in blackcurrant by infection with some nepoviruses, cucumber mosaic cucumovirus and alfalfa mosaic virus ([1]; A.T. Jones, unpublished information).

Blackcurrant reversion, together with some other mite-transmitted virus-like agents[3], have been an enigma in science for over 50 years because much research has failed to identify the causal agents of these diseases. The seriousness of reversion disease in blackcurrant has initiated many attempts to identify its causal agent. However, earlier claims that it was a mycoplasma-like agent[4] or a potyvirus[5] have not been

Figure 4 Conspicuous chlorotic line-patterns on the leaves of reverted blackcurrant plants.

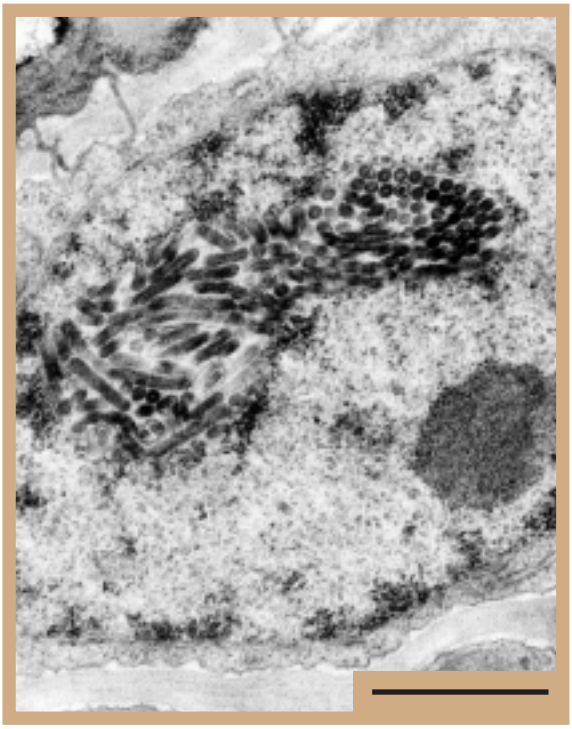

Figure 5 Rhabdovirus-like particles in the nuclei of xylem parenchyma cells of blackcurrant affected with the R form of reversion disease. Bar = 1µm.

substantiated by other workers ([1]; A.T. Jones and I.M. Roberts, unpublished data). Recent work at SCRI identified several dsRNA species in reverted blackcurrant plants that were not present in virus-tested mother stock plants, but further work has shown that some of these dsRNA species are present in some older blackcurrant cultivars that are free from reversion disease. No specific dsRNA species were therefore consistently associated with the disease (S. Cox, A.T. Jones and M.A. Mayo, unpublished data). More recently at SCRI, extensive studies were made to identify the possible morphological structure of the reversion agent and/or changes in cell ultrastructure associated with it, by using electron microscopy to examine ultrathin sections of different organs of reverted blackcurrant plants and of gall mite vectors from such plants. No such structures were observed in mites[6] but, in a very small proportion of parenchyma cells of vascular tissue of a few plants affected with the R form of the disease, rhabdovirus-like particles *c.* 65-80 x 215-485nm were detected (Fig. 5)[7]. Such particles have not been reported previously from *Ribes* and we detected them in only three of seven plants affected with the R form of reversion, but not in any of five plants affected with the E form. It is not clear if the failure to detect these particles in some plants is

Figure 6 Systemic chlorotic flecking in *Chenopodium quinoa* two weeks after mechanical inoculation with BRAV.

due to their absence, or the difficulty of detecting them due to their very low frequency of occurrence in cells. However, as morphologically similar particles were also observed in vascular cells of gooseberry that is reported to be immune to the reversion agent[1], it suggests that these rhabdovirus-like particles are unlikely to represent the causal agent of reversion disease, at least not on their own.

This lack of knowledge of the disease agent has prevented the development of tests for its rapid detection, so that the test for infection in plants today is still dependent on traditional graft-inoculation of test material to sensitive blackcurrant cultivars that was developed over 40 years ago. Furthermore, because the reversion agent is erratically distributed in infected plants (Fig. 3), it is necessary to test material from several branches of the same test plant and to await symptom development for up to 2 years[1]. However, this impasse in identifying the causal agent of reversion disease may have been overcome following work in Finland that isolated, with great difficulty, a sap-transmissible virus from a rooting cutting of a black-currant plant affected with the R form of the disease, and which developed pronounced chlorotic/yellow line patterns in newly produced leaves (Fig. 4). This virus was mechanically transmitted to *Chenopodium quinoa* (Fig. 6) and, from this host, to a range of other herbaceous test plants, that allowed it to be cultured, purified, partially characterized, and an antiserum to it produced. In collaborative studies, the virus was found to have isometric particles *c*. 27 nm in diameter (Fig. 7) that sedimented as two nucleoprotein components. Virus particle preparations contained a major protein species of *M*r 55kD and two major RNA components of *c*. 6700 and 7700 nucleotides, each with poly-A tails. Several of these properties are shared by members of the proposed sub-group 3 of nepoviruses, but the virus was serologically unrelated to 9 possible members of this sub-group or to 5 other nepoviruses, or putative nepoviruses tested. However, the deduced nucleotide sequence of part of the 3' end of one of the viral RNA species contained short regions of homology to the 3' terminal sequences of RNAs 1 and 2 of cherry leaf roll nepovirus, and to the RNA 2 of cowpea mosaic and red clover mottle comoviruses; apart from these short regions, the partial viral sequence is distinct from those reported for other viruses. The virus therefore appears to be newly described.

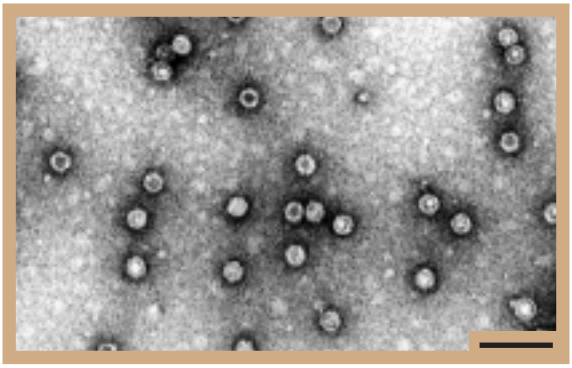

Figure 7 Electron micrograph of a purified preparation of BRAV particles stained in uranyl acetate, pH 3.5. Bar = 100nm.

The isolation and partial characterisation of this virus have provided the materials for its rapid and sensitive detection in plants. Primers were designed from the known partial sequence to amplify a 210 bp region of the cDNA of the virus RNA using an immuno-capture-reverse transcriptase-PCR (IC-RT-PCR) protocol. This technique has given the necessary sensitivity and reliability to assay this virus in a wide range of *Ribes* plants infected with different viruses or virus-like agents, and to draw some conclusions on its possible involvement, or otherwise, in known diseases of *Ribes*. Data from such analyses have shown a very close association of the virus with reversion disease in plants. It was detected by IC-RT-PCR in plants showing symptoms of the E or R forms of the disease (Fig. 8), whether they were of Finnish, Scottish or New Zealand origin and also in gall mites collected from reverted plants. It was also associated with the field spread of reversion into initially healthy blackcurrant plants and was detected in initially healthy blackcurrant plants on which gall mites from reverted plants

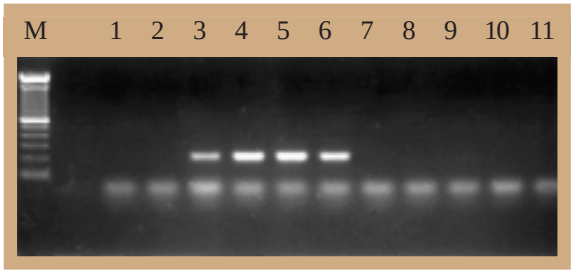


Figure 8 IC-RT-PCR amplification of the 210 bp cDNA fragment of BRAV RNA from nucleic acid extracts from individual blackcurrant bushes. Lane 'M' contains a 100 bp mol. wt ladder (Gibco BRL). Samples are (lanes in parenthesis), healthy plants of the blackcurrant cultivars Ben Alder (1, 7), Ben Lomond (2, 11), Ben Nevis (8), Ben Tirran (9) and Ben Sarek (10), and unnamed cultivars affected with the R (3, 4) and E (5, 6) forms of reversion disease.

had fed experimentally. However, the virus was not detected by IC-RT-PCR in healthy *Ribes* plants, in *Ribes* plants free from reversion but affected by three other distinct virus-like diseases of *Ribes*, or in plants infected with arabis mosaic, strawberry latent ringspot or raspberry ringspot nepoviruses. In assays of individual buds from branches of a plant of Ben Lomond blackcurrant naturally infected in the field with the E form of reversion, the virus was detected in only 50% of the buds, ranging from 28-60%, depending on the branch assayed (Fig. 9). There was no clear pattern of distribution but a large proportion of the cluster of buds near the tips of some branches assayed were infected, suggesting that this region might be the best to sample to detect infection in plants. This erratic distribution of the virus is in keeping with the known erratic distribution of the reversion agent in plants.

Taken together, these data suggest strongly that this virus may be the causal agent of reversion disease and it is tentatively called, blackcurrant reversion associated virus (BRAV). However, unequivocal evidence that BRAV is the causal agent of the disease depends on the ability to fulfil Koch's postulates by infecting healthy blackcurrant plants with the purified virus and reproducing the disease. Currently, technical difficulties prevent this because *Ribes* plants are very difficult to infect with virus by mechanical inoculation, due presumably to the high levels of tannins and polyphenols in leaves. Also, the development of reversion symptoms in inoculated blackcurrant plants may take up to 2 years[1] Despite the absence of this final proof, our finding of a very close association of BRAV with reversion disease and the development of a sensitive

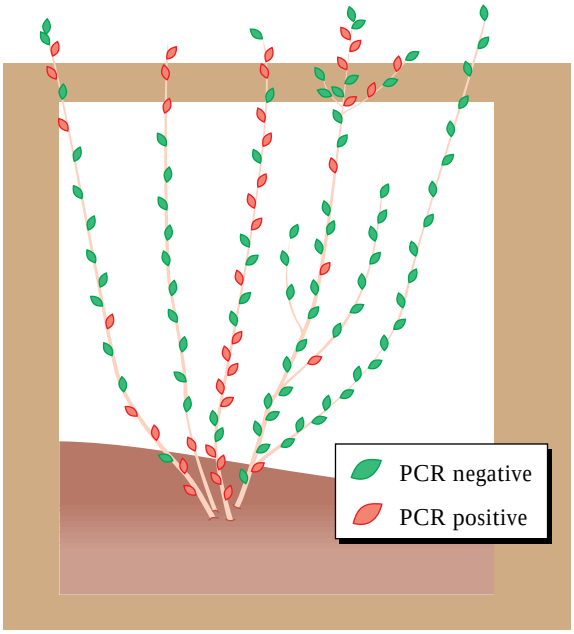


**Figure 9** Diagrammatic representation of the occurrence of BRAV in assays on individual buds of branches of a reverted plant of Ben Lomond blackcurrant.

protocol to detect it in *Ribes* tissue, are major steps forward in the possible identification of the causal agent and a rapid means of diagnosing it in plants. They also provide a means to study, in more detail, the relationship of BRAV with its plant host and possible mite vector.

Finally, if BRAV is found to be the causal agent of reversion disease, and if the remaining sequence of its RNAs confirm its status as a nepovirus, then it poses interesting questions regarding the transmission of the majority of nepoviruses that have no known vector. In the light of our work with BRAV, it may be opportune to examine the possibility that mites may also act as vectors for some of these nepoviruses for which the mode of transmission is not known.

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