FRESH NOTES
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# Galls in apple: What do, and don't we know?

## What is the extent of the gall issue in the apple industry?

When first reported during autumn 2022, three nurseries and one rootstock, G.778, were affected. Since then, galls have been found at most nurseries – there are a couple of exceptions – and in addition to G.778, which is still the most affected, galls have also been found on the three most common M9 clones and on G.969. Galls have also now been found on G.778 trees planted in 2021 and 2020 and M9 trees planted in 2021 and 2017. Hence it would appear that galls – or the organism that gives rise to galls - have been affecting nursery material of G.778 and M9 for at least a couple of seasons. Figures 1 and 2 show examples of nursery trees with galls.

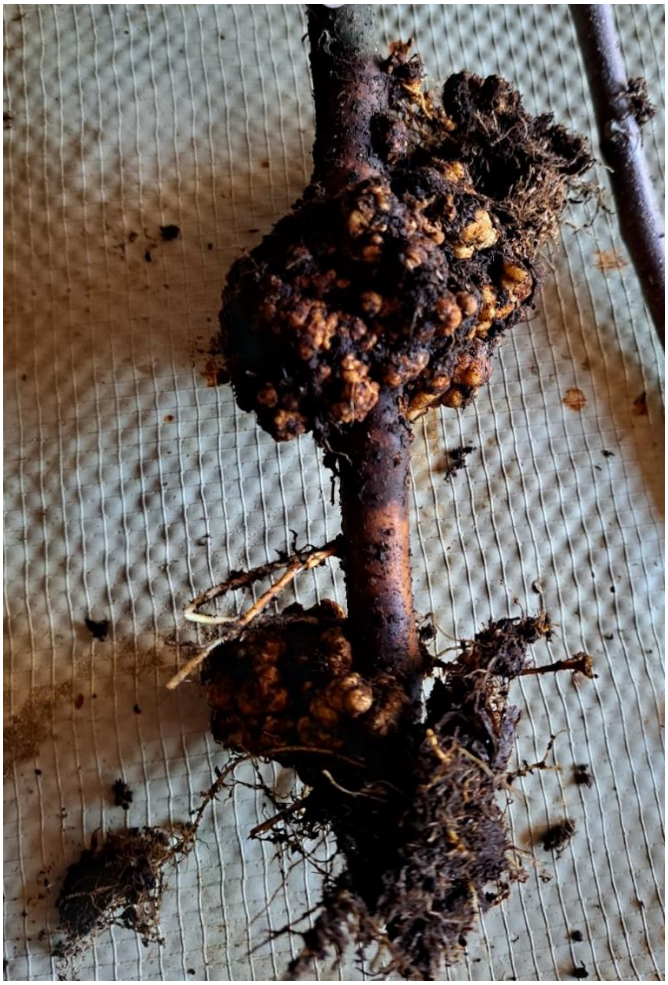

*Figure 1: Galls on an apple nursery tree on M.9 rootstock. The root system is poorly developed, likely due to the presence of the uppermost galls that are causing girdling. (Photo: K Theron)*

*Figure 2. The root system of an apple nursery tree on G.788 showing gall formation on the shank and on roots. In this case, the galls did not cause girdling and some strong roots are present. (Photo: R Heyns)*

## Why have the presence of galls gone under the radar up until now?

There are a couple of possible reasons which alone or in combination could have contributed to the presence of galls going under the radar. These may include:

- Growers (and nurseries) were not on the lookout for galls,
- It is mostly uncertified material that has been affected and therefore it was not picked up during scheme inspections. Theoretically, the inspectors of DALRRD also inspect uncertified trees but due to capacity constraints, in practice this doesn't happen to the necessary extent,
- Crown gall occur at very low (0.1 – 1%) levels in apple nurseries globally. Every now and again, for reasons that are not very well understood but probably relating to growing conditions, infection may increase to more noticeable and problematic levels – the American Phytopathological Society's Compendium of apple and pear diseases and pests mentions levels of up to 80% during an epidemic.

## What is the cause of the galls?

Despite not having been able to identify the causative organism to date, we believe the galls to be caused by a pathogenic strain of *Agrobacterium tumefaciens*, which means that we are dealing with crown gall. The crown gall pathogen is unique since it can insert its own genes, specifically oncogenes (auxin and cytokines) and food source production genes (opines), into the plant's genome. The oncogenes result in excess cell proliferation and gall formation.

*Figure 3. Crown gall should not be confused with burr knots, a mass of adventitious root initials that develop on the aerial part of the tree. Certain cultivars and rootstocks like M.7 and M.9 are more prone to develop burr knots. Rootstocks that are more prone to develop burr knots root more easily. However, since burr knots can provide an entry point to disease and pests, and can also weaken the trunk, modern apple rootstock breeding programmes like the Cornell Geneva programme have been selecting against the presence of burr knots. (Photo: W Steyn)*

## Why haven't we been able to identify the pathogenic A. tumefaciens strain that is causing our galls?

Two different laboratories are busy with their third set of analyses. So far, they have been unable to isolate the pathogenic, i.e., disease causing, *A. tumefaciens* strain despite isolating numerous non-pathogenic *A. tumefaciens* strains. While much easier in other crops, it is notoriously difficult to isolate pathogenic *A. tumefaciens* from apple. Hortgro has recently confirmed this with a plant diagnostic laboratory in the USA where apple crown gall samples are received on a more regular basis than in South African laboratories. The reason for the pathogen being difficult to identify in apple galls is that apples somehow deactivate and extrude the pathogenic *A. tumefaciens* strain after gall formation has commenced. This could be due to apple galls being unique in that they lack the ability to produce the pathogen's food source (opines), unlike in stone fruit galls. Furthermore, the non-pathogenic *A. tumefaciens* strains are likely able to outcompete the pathogenic strain after the commencement of gall formation in apple since they do not have to invest energy in hijacking the plants genetic material and battling its defenses. The net effect is that by the time that galls become noticeable, and testing is done, the pathogenic strain is not present anymore.

A level of caution is needed in making a crown gall diagnosis in the absence of isolating the pathogen. Mark 7 apple rootstock, for example, is very sensitive to crown gall, but also produces copious calluses. These calluses can be mistaken for galls. However, the widespread incidence and the way in which the galls have been manifesting in our industry strongly suggests that we are in fact dealing with a crown gall outbreak.

## Did production in pots and tissue culture propagation play a role in the presence of galls?

There is no evidence that links production in pots and tissue culture to crown gall. In fact, since *A. tumefaciens* is omnipresent in soil, soilless cultivation is seen as one of the main ways to prevent or at least lower the incidence of crown gall and other soilborne diseases in nursery plants. It does seem though that in the presence of *A. tumefaciens* the potted environment can increase the incidence and severity of gall formation. Note though that the presence of soil in the potting mix can serve as a source of infection. Some nurseries that largely grow tissue cultured G.778 and M9 plants in pots without a layer bed step so far had a zero incidence of galls.

It has been suggested that the G.778 and M9 trees showing gall formation had a tissue culture origin, which may point to a latent infection of tissue culture material at source or subsequently at some early point during the propagation process. While this cannot entirely be ruled out, some evidence suggests otherwise. For starters, nearly all the plants with galls have been grown from liners. These liners were obtained from stool beds established from tissue culture plants. Hence, these plants have been in contact with soil, which would be the more likely source of infection. Note again that some nurseries producing tissue culture plants in pots in sterile non-soil medium have zero incidence of crown gall.

## Are G.778 and M9 more susceptible to crown gall?

There is generally very little information available on the susceptibility of new rootstocks to crown gall. Apple rootstocks do differ in their susceptibility to crown gall. While it remains to be proven – Hortgro intends to fund a project assessing rootstock susceptibility – it could be that G.778 is more susceptible to crown gall. M9 is seen as more susceptible than some other rootstocks, but not as susceptible as Mark 7 (not to be confused with M7, which is a different rootstock). The M9 purported susceptibility to crown gall has not prevented it from becoming a global apple rootstock. We should not write off certain rootstock before we have the facts. The size of crown gall epidemics is known to be influenced by rootstock susceptibility.

## What are some of the environmental factors associated with high crown gall incidence in apple nurseries?

The following factors seem to be associated with higher levels of infection:

- Wounding, which include mechanical damage, the emergence of new lateral roots from the stem, nematode and insect damage, chemical damage/burn to roots, as well as budding and grafting, is essential for infection,
- Heavy, poorly drained soil seems to suffer greater levels of infection,
- Optimum temperatures for infection are 20ºC and above,
- There is much conjecture about the effect of soil pH with different sources stating lower incidence in acidic and alkaline soils.

## How does crown gall affect apple tree growth?

The impact of crown gall on tree performance may differ depending on the severity and location of the galls on the root system. The presence of galls high on the rootstock above the root system and circumventing the trunk may cut off exchange of water, nutrients and assimilates between the shoot and root system – the tree is in effect girdled and growth will be stunted. Similarly, the presence of many galls can disrupt the vascular tissues to such an extent that trees are stunted (Figures 4 and 5). A nursery tree covered in galls with none or only a few viable roots can be expected to perform worse than a tree with very few galls and a relatively healthy root system. Conversely, the presence of a gall at the proximal end of the rootstock below the roots will not cut off shoot and root transport and may have less of an effect (Figure 6). However, these trees would still be an inoculum source for new infections to occur post-plant.

Trees are generally affected most in the year of establishment or shortly thereafter– the lifting/planting of trees can cause wounding and result in further gall formation. Once the tree has established, and, crown gall may become less of an issue, provided there is no further damage to the root system. It has been reported though that severe crown gall infection may stunt growth and reduce fruit size in mature trees. Heavy levels of gall formation present a continuous drain on the plant's resources and impedes nutrient, hormone, and water supply. This causes the gradual decline of mature trees over time. The stress caused by tumors and the weakening of trees over time also make them susceptible to other bacterial, fungal and insect infestations. On the other hand, according to the Compendium of apple and pear diseases and pests, some studies have also found little to no effect of crown gall infection on tree performance. This is however most likely due to environmental conditions not being conducive to disease development post-plant or the planted rootstocks were not highly susceptible to crown gall.

*Figure 4. One-year-old apple tree on G.778 rootstock showing significant gall formation and little root growth. (Picture: A Pretorius).*

*Figure 5: One-year-old apple tree on G.778 rootstock showing excessive gall formation. Tree growth was severely stunted, and the orchard had to be grubbed. (Photo: R Heyns)*

*Figure 6. One-year-old apple tree on M.9 with healthy roots above a gall on the lower shank. (Photo: K Bradley)*

## Can crown gall be treated in apple?

Crown gall in stone fruit is prevented by treating plants with K84, a strain of *A. radiobacter* that produces an antibiotic, agrocin 84. Note that K84 is not a curative but a preventative treatment. Not all *A. tumefaciens* strains are sensitive to agrocin 84. Du Plessis et al. (1985) found that of 82 pathogenic strains of *A. tumefaciens*, 36% and 15% were respectively sensitive and moderately sensitive to agrocin 84 *in vitro.* Although agrocin 84 is the main mode of action for biological control of crown gall, other mechanisms are also at play, but these are less effective. Consequently, in the presence of agrocin 84 resistant pathogens, the efficacy of biocontrol can be reduced by more than 70%, which may not be commercially acceptable if a high level of disease or latent infections (pathogen present but no symptoms yet) are present in nursery stock. International literature suggests that K84 is generally not effective in preventing crown gall in apple. However, a Spanish study found good efficacy against a Spanish strain of crown gall in apple using K84 in pot tests. To be sure, we need to assess the effectiveness of K84 on the local pathogenic strain causing the galls in apple, once identified. In other crops, non-pathogenic strains other than K84 have proven effective against crown-gall. The potential thus exists to identify such a strain on apple for use as a biological control agent in South Africa. This, however, will take a substantial research effort.

Chemical treatments (creosote, antibiotics, copper oxychloride, etc.) have generally proven either ineffective or not feasible. Soil fumigation has yielded variable results – generally soil fumigation is not considered as an effective means to control crown gall.

## What should growers do about orchards with crown gall?

Crown gall is ubiquitous in soil and a very low level of infection in some orchards, as in nurseries, would not be unexpected. If an orchard is growing well, there is no reason to remove it. One might, however, want to let the soil rest for 4 to 5 years before replanting. Note though that it is for all practical purposes impossible to eradicate pathogenic *A. tumefaciens* from a soil once established.

## Should growers plant apple trees with crown gall?

Under the deciduous fruit plant certification scheme, trees with crown gall will not pass inspection and cannot be sold or distributed as certified trees. Only DALRRD can prevent the sale and distribution of uncertified material. In principle, the planting of diseased trees is not something that we could ever condone. Best practice is to discard any plant material showing crown gall symptoms.

Planting trees with galls will introduce the pathogenic strain to soil where it will remain indefinitely. This is because pathogenic *A. tumefaciens* strains can live as a saprophytes and can also infect various weed species. Removal of galls will not cure the tree.

Nothing prevents growers from planting uncertified trees, but they need to be cognizant of the inherent risk of planting such trees. Various factors will need to be considered, for example, the severity of gall formation. A planting decision should be taken in consultation with the nursery supplying the trees and preferably also a technical consultant. Growers will need to realize that the risk of poor performance of trees after planting will be theirs and the nursery and technical consultant cannot afterwards be blamed if a joint decision was taken to plant infected trees. Growers also need to realize that once present in trees and in the soil associated with these trees, it is nearly impossible to get rid of the pathogen.

## What has Hortgro been doing to date to address the crown gall problem?

Hortgro became involved once it became clear that the galls are not limited to a single rootstock at three nurseries and that we are most likely dealing with crown gall. Prior to that, for a short period, the rootstock license holder, SAPO Trust, dealt with the issue in consultation with Hortgro. Hortgro subsequently took over the coordination of a group of nursery representatives, researchers and technical advisors brought together by SAPO Trust.

PlantSA inspectors were alerted to the presence of galls and asked to assess the presence of galls on G.778 rootstock at all the nurseries they've inspected – this was before the wider presence of galls on M9 was known.

After the first tests at two laboratories, i.e., the Plant Disease Clinic at the Department of Plant Pathology at SU and the ARC diagnostic service at Infruitec-Nietvoorbij, failed to isolate the causative organism, Hortgro funded a further two rounds of testing – the third round is still in progress. The testing is quite laborious and time consuming, but it is important that we isolate the causative *A. tumefaciens* strain for further actions and research that need to be undertaken. Numerous consultations were had with Prof Adele McLeod and Sonja Coertze at the Department of Plant Pathology at the SU and Dr Yolanda Petersen at the ARC and these plant pathogen experts have in turn been consulting with their international networks in order to optimize the test. Our understanding of crown gall has mostly been developed based on these consultations and on

extensive literature searches. It must be noted thought that there is a global shortage of plant bacteriologists and particularly so in terms of people specializing on crown gall in apple.

Hortgro has sent out two communications alerting growers to the presence of galls on nursery trees and also via PlantSA a communication to nurseries informing them that we are considering the galls to be caused by *A. tumefaciens* and that it should be treated as such.

Meanwhile, Prof Karen Theron has been visiting various nurseries to try to identify factors associated with the incidence and severity of the galls.

## What are the next steps?

Once pathogenic *A. tumefaciens* is present in soil, it is very difficult to eradicate. We propose the following actions:

- Soil fumigation has proven largely ineffective so planting nursery blocks at contaminated sites should be prevented. Soils and water should be tested for the presence of the pathogen.
- Nursery material (medium/pots/etc.) and all implements need to be sterilized and in soil operation moved to new localities.
- Wounding should as far as possible be prevented or kept to a minimum. Nursery practices should be adjusted accordingly.
- Diseased material should preferably be destroyed by burning. *A. tumefaciens* can survive on organic matter for several years from where it can serve as a source of contamination of adjacent soil and water sources. Diseased material should therefore not end up in the soil or on compost heaps.
- Planting material, i.e., tissue culture plants and liner beds need to be tested for the latent presence of the pathogenic *A. tumefaciens* strain, once this strain has been identified. Growing media and water sources should also be tested.
- The range of rootstocks available to growers should be screened for crown gall susceptibility.
- The efficacy of K84 and other non-pathogenic *Agrobacterium* strains should be evaluated for their biological control potential.
- Trees with different levels/severities of crown gall infection should be planted in soil where the pathogen is now known to occur – not in clean soil – and the progression of the disease and its effect on the trees followed over several years.