

Interim Report on G.213 Graft Union Syndrome

Author: Gennaro Fazio – Breeder CG Rootstocks, USDA ARS

Executive Summary

- A variable percentage (according to location) of trees grafted on G.213 apple rootstock has been collapsing 2-3 years after planting and some rootstock scion combinations are not thriving at the nursery stage and continue to slow down during the first few years in the orchard.
- The syndrome has been attributed to and is highly correlated with callous tissue formed at the time of budding/grafting – it is unique to G.213 genetics associated to hormonal balance.
- The callous tissue is sensitive to the formation of proto-burr-knots which stay internal to the bark and cause growth of inordinate (chaotic) parenchymatic tissue which interferes in the long term with the proper vascular activity of the graft union thereby physiologically “girdling” the trees.
- Variables affecting the incidence of syndrome include:
 - Scion variety
 - Type and timing of grafting/budding – **IMPORTANT: dormant grafting or budding seems to decrease the problem significantly to a very low level (less than 2%).**
 - Presence of wet conditions near the graft union exacerbated by protecting barriers (cartons, plastic nets or anti-vole ribbons).
- While initially the process of micropropagation was thought to be involved, the development of the same syndrome in layer bed produced material has resulted in the rejection of that hypothesis.
- While the syndrome weakens the trees (and roots), all of the observed deaths (collapses) were due to secondary infections, rabbit (or vole) browsing or insect infestations that penetrate the cracked bark.

Background

In Europe, a variable percentage of trees grafted on G.213 apple rootstock has been collapsing 2-3 years after planting and some rootstock scion combinations are not thriving at the nursery stage and continue to collapse during the first few years in the orchard.

Specific scions observed in this trip that show problems are WA-38, UEB, Innored, while some Gala, Fuji, Golden and Red Delicious do not seem to be affected.

Visits included field trials in the Fragsburg and Laimburg research station - WA38, UEB on G213 and other trials. They also included commercial plantings of G.213 with local scion varieties including Innored, Gala and UEB in various regions of northern Italy.



Collapsed tree on G.213 at the Verona location of GRIBA Nursery.



Observations in Italy - Description of the symptoms and plant material

In Italy, there were no layer beds, therefore all the affected G.213 is plant material that had been micro-propagated. Some of the material was delivered during the fall and some was delivered during late spring or summer.

Some of the plant material was in the form of freshly acclimated plants and some had gone through a dormancy (leafless) period.

The material was grafted with many varieties including Gala, Golden, UEB, WA-38, Fuji, Red Delicious, Innored. The percentage of the symptomatic plants varied according to variety, timing budding/grafting, stages of acclimation of plants (fresh vs dormant) and location in the field. Symptoms begin in the nursery as bulges (usually two) below the graft union which then progress in growth in the orchard producing cracked inordinate bark with microscopic and larger breaks, which can be colonized by insects, bacteria and fungi causing rots and death of the tree. The growths also seem to dwarf the trees significantly.



Observations in Brazil - Description of the symptoms and plant material

Affected G.213 plant material originated from stool bed (several nursery locations) or micropropagation delivered green to several nurseries. Some of the material was delivered during early spring into mid summer. All the material was grafted by whip and tongue method while rootstock was actively growing with graftwood that had been maintained in refrigerator.

The material was grafted with mainly two varieties: Gala and Fuji.

Symptoms seem to begin in the nursery as callous tissue between the rootstock and scion tissues which then progresses in growth in the orchard producing cracked inordinate bark with microscopic and larger breaks, which can be colonized by insects, bacteria and fungi causing rots and death of the tree. The growths also seem to dwarf the trees significantly – probably by inhibiting good phloem flux and inhibiting straight xylem vessel formation.

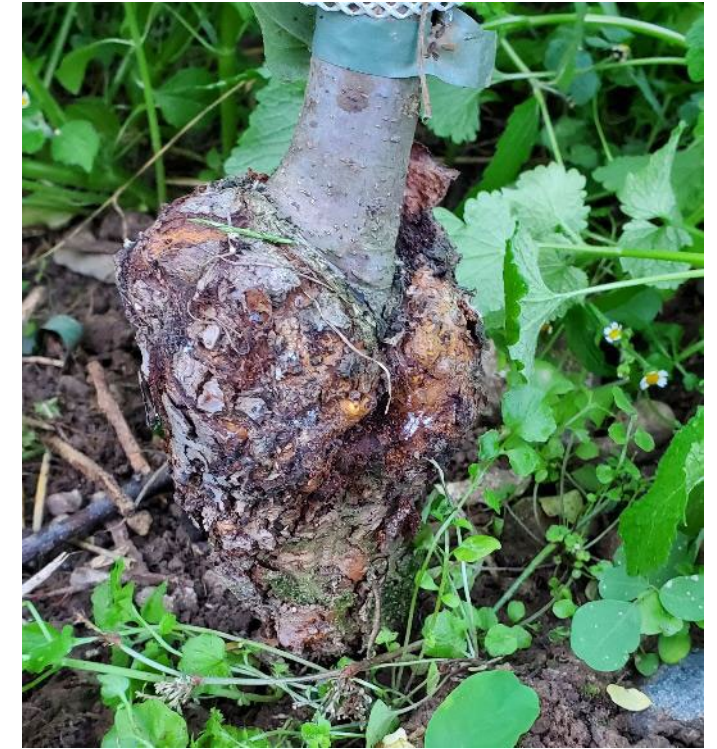


Additional information on symptomatology

- Symptoms appear to be specific to certain scion-rootstock combinations.
- Symptoms seemed to appear in larger percentages with rootstocks received “fresh” by micropropagation: which means that rootstock tissues were likely still in a “juvenile” phase with totipotent cells in areal tissues ready to differentiate into different tissue types.
- The presence of wetter conditions and protective graft union guards increased the rate of this syndrome.
- In addition, the growth rate of the trees after planting seems to be a factor too.
- Same type of trees being planted a few kilometers apart (Dalpiaz vs Bonifica Tuenno) differing the first showing significant symptoms under wetter soil, full rootstock guards, normal management (more soil fertility) and less symptoms under organic management (drier, less soil fertility) and slower growth.



Normal Graft Union Gala scion on G.213 rootstock (South Tyrol)



Abnormal Graft Union UEB scion on G.213 rootstock (South Tyrol)

Introduction to burr-knots in apples

- Burr-knots are adventitious areal root formations that are found in some apple tree varieties and many of the traditional old-style rootstocks like Malling 9 (M.9) and Malling 26 (M.26)
- There is a genetic predisposition to these growths and in the Geneva apple rootstock breeding program it has been selected against **such that our rootstocks do not produce them – including G.41, G.935, G.202, and others.**
- Burr-knots formation is a negative trait for field performance because it is a source of colonization for borer insects which then girdle the tree killing it or let in fungi and bacterial that cause rots.



Internal symptoms: what are the bulges made of?



Cutting into the abnormal growths reveals the preponderance of small round inclusions surrounded by chaotic interstitial parenchymatic tissues. Of all the different hypothesis about what these round inclusions are, the most that makes sense is that they represent proto adventitious root formations (proto burr knots) that never emerge from the bark and remain inside. The hyperplasia inside the bark is likely caused by cytokinin production by the proto-root tips and with nowhere to go, the cells nearby continue to divide and grow inordinately. These formations appear very early a few weeks after the grafted bud starts growing in the nursery. These images were collected during my trip.

Preliminary observations

1. The syndrome is independent of propagation type as in the case of Brazil, G.213 plants were derived from both stool (layer) bed propagation and micropropagation.
2. We are unsure if green tissue from micro-propagated plants increases the percentages of affected plants. It may be possible that micro-propagated plants that had not been allowed to go dormant before grafting can produce more callous (the sensitive tissue) compared to plants that were allowed to go dormant. THIS IS AN AREA THAT NEEDS INVESTIGATION.
3. It possible that the juvenile status of the rootstock reacts with endogenous hormones at the graft union spurring the formation of lots of callous and eventually root primordia which then cause production of cytokines by the proto-root tips stimulating the hyperplasia below the graft union. The physiological and endogenous hormonal status of the scion variety must be different (Gala vs WA-38 – AKA Cosmic Crisp) to influence significantly the formation of the proto-roots.
4. The hyperplasia inside the trunk caused micro breaks in the protective bark layer which can be quickly colonized by insects, fungi and bacteria to finally cause an overwhelmed confused tissue to be colonized by rot and the tree to collapse.
5. It is possible that exogenous hormonal treatments in the nursery phase, excess wetting or certain types of root inducing phytophthora may exacerbate the formation of these “ingrown” burr-knots.



Probable fungal formations (I did not have fungal stain with me) in cracked bark and holes made by insects.



The beginning of an internal burr knot formation on the rootstock shank near the graft union where the dark tissue has been stained with iodide solution.



Some of the insects found under the irregular bark formations.



The initiation of the chaotic cracks in the bark which attract insects and fungi.

Initiation of root primordia



Root primordia and accumulation of starch is forming on the rootstock next to the graft union. The staining is done by iodide, meaning starch is accumulating in that region. This tissue likely originates as callous tissue between scion and rootstock



What tissue is producing the problem?

The problem seems to originate from the callous tissue formed at the time of the graft. Probably a mix of G.213 rootstock cells and Gala cells, Gala being more sensitive to making burr knots. We followed the tissues in these cutout of the graft union and found that on the side where the rootstock whip is up (BLUE) there is no formation of problem tissue, but on the other side made mostly by Gala cells and a possible mix of callous cells from G.213 and Gala (RED) the problem tissue is very pronounced. In three-dimensional space the problem tissue make occupy more than $\frac{3}{4}$ of the graft union.

Material courtesy of Rufato Lab



Growths prevent proper healing of rootstock cut wound

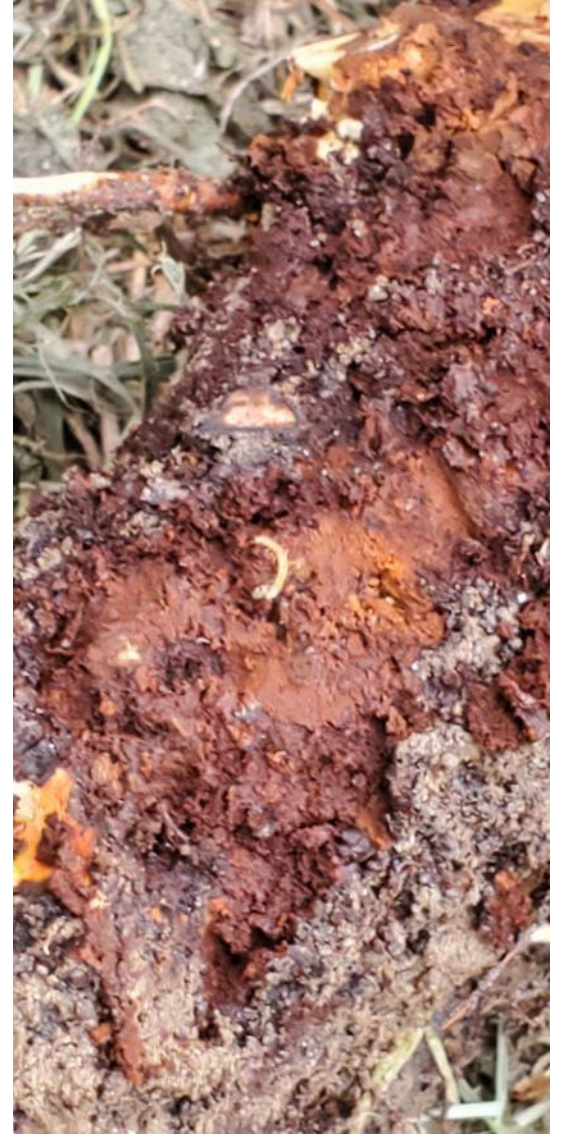
The non-healed cut wound seems to be the common denominator of the rot that happens later on. It is likely that a normal chain of events that lead to a proper healing and encasement of the wound is disrupted by the hormonal confusion in the region. Xylem and phloem are affected.



Not all the collapsed trees are due to the hyperplasia, found evidence in Europe and Brazil of plenty of critters that feast on the exposed rootstocks letting in bacteria and rot causing fungi and girdling the tree



However, even in perfectly formed graft union we find evidence of insect colonization that leads to rot



These two pictures of grafts in Brazil show the very different behavior of the callous tissue compared to regular stem. These stocks are from layer bed.



Budding/Grafting in Apples

Chip bud

Whip/tongue

Saddle



Source: Royal Horticultural Society



Source: Adelaide Kitchen Gardeners



Source: Chris Vernon

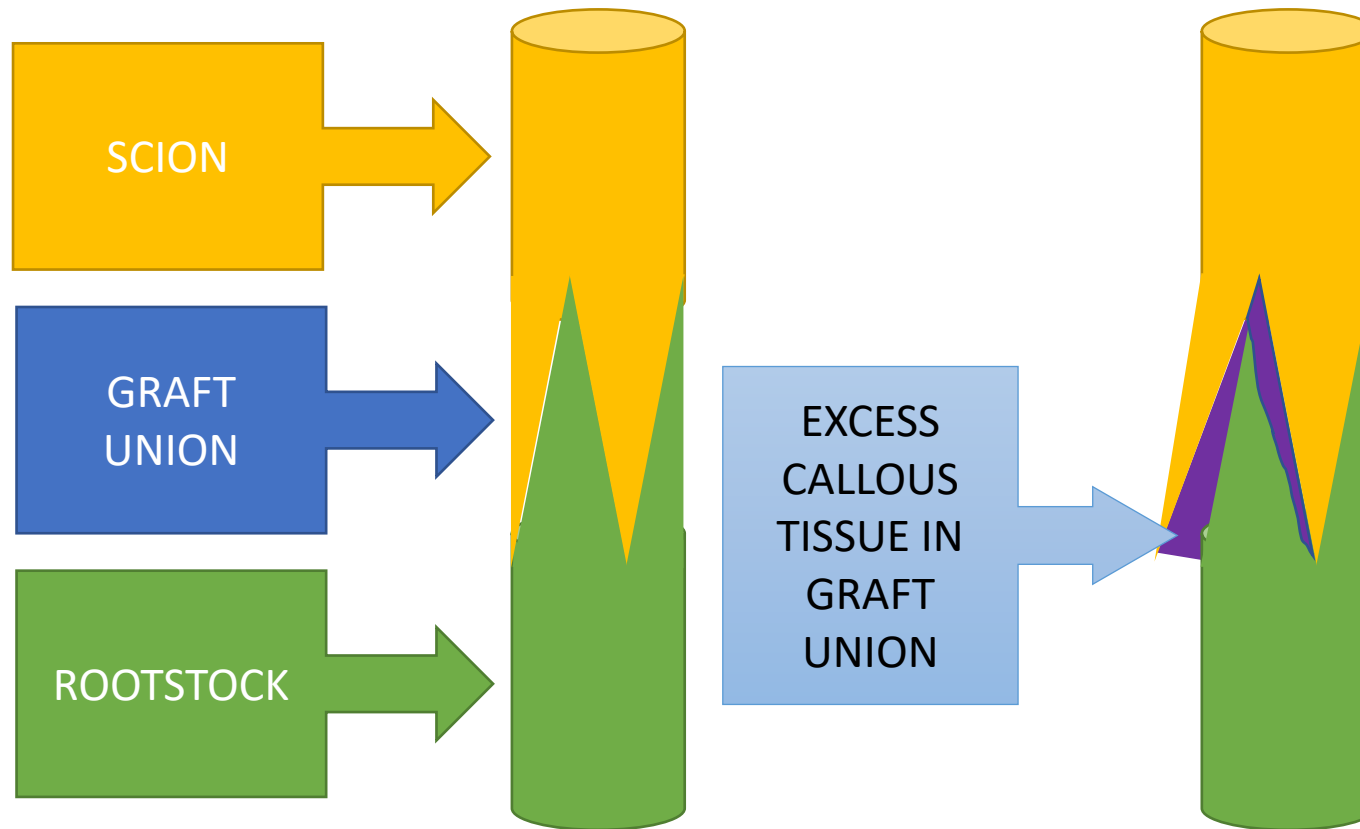
Model hypothesis

- The previous slide showed near perfect buds or grafts.
- The next few slides show different models of how the callous tissue takes over the graft union.
- More callous is correlated with more problems.

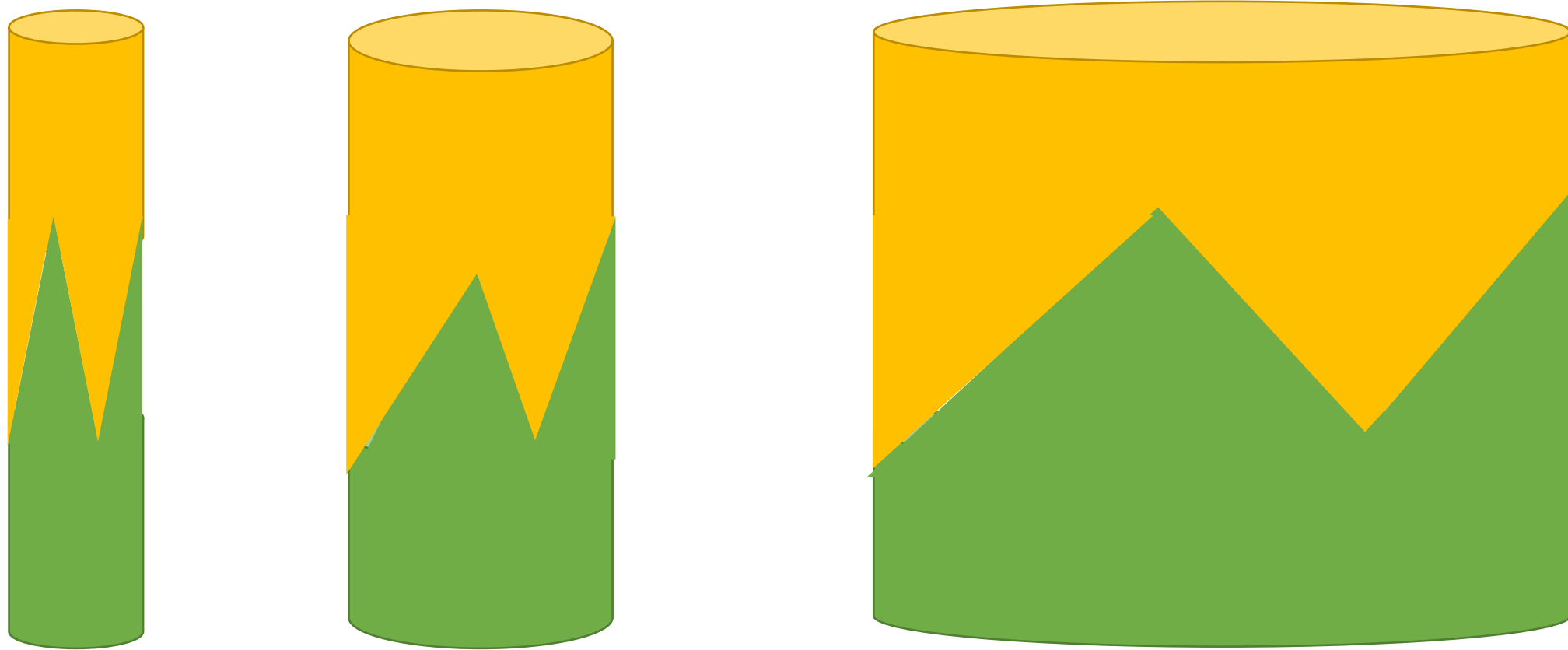
Whip and Tongue Grafts

Tight graft union

Uneven graft union

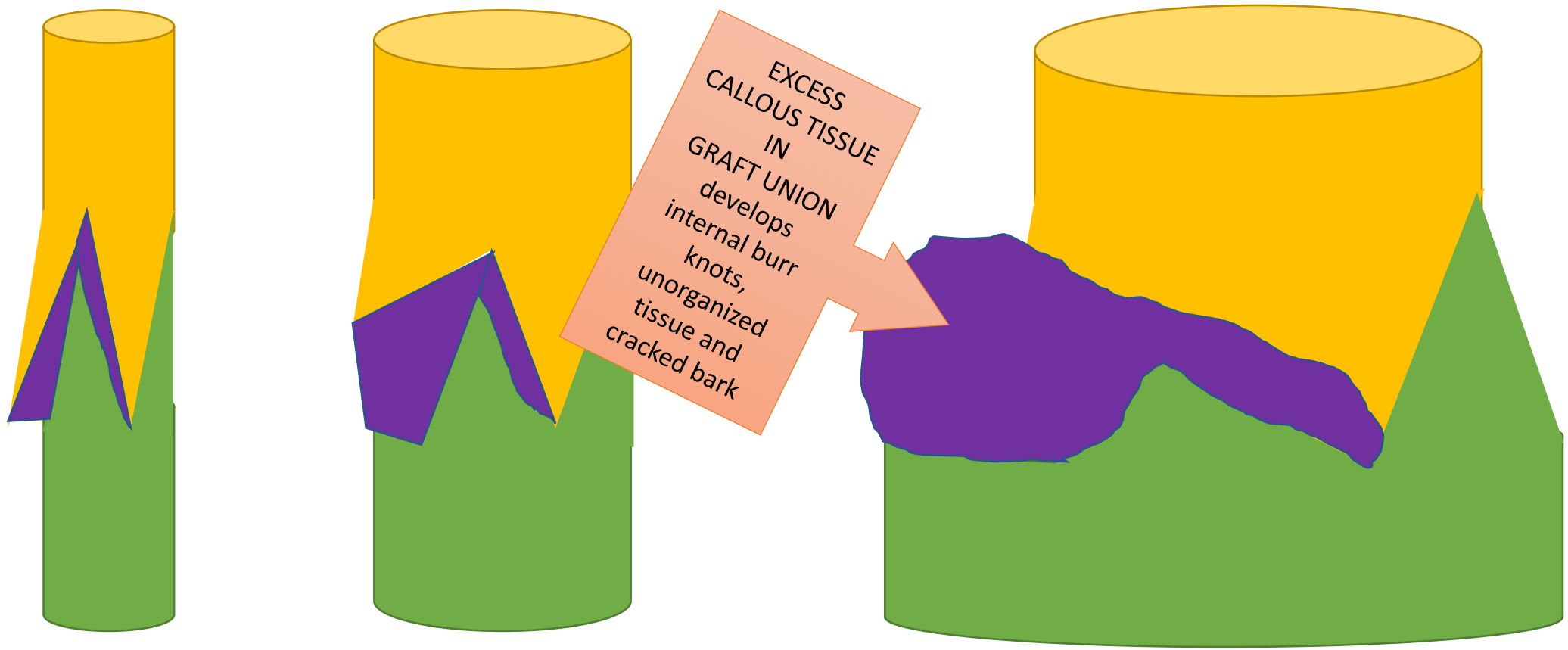


G.213 ideal graft union as seen with Cripps Pink, Fuji and other scions



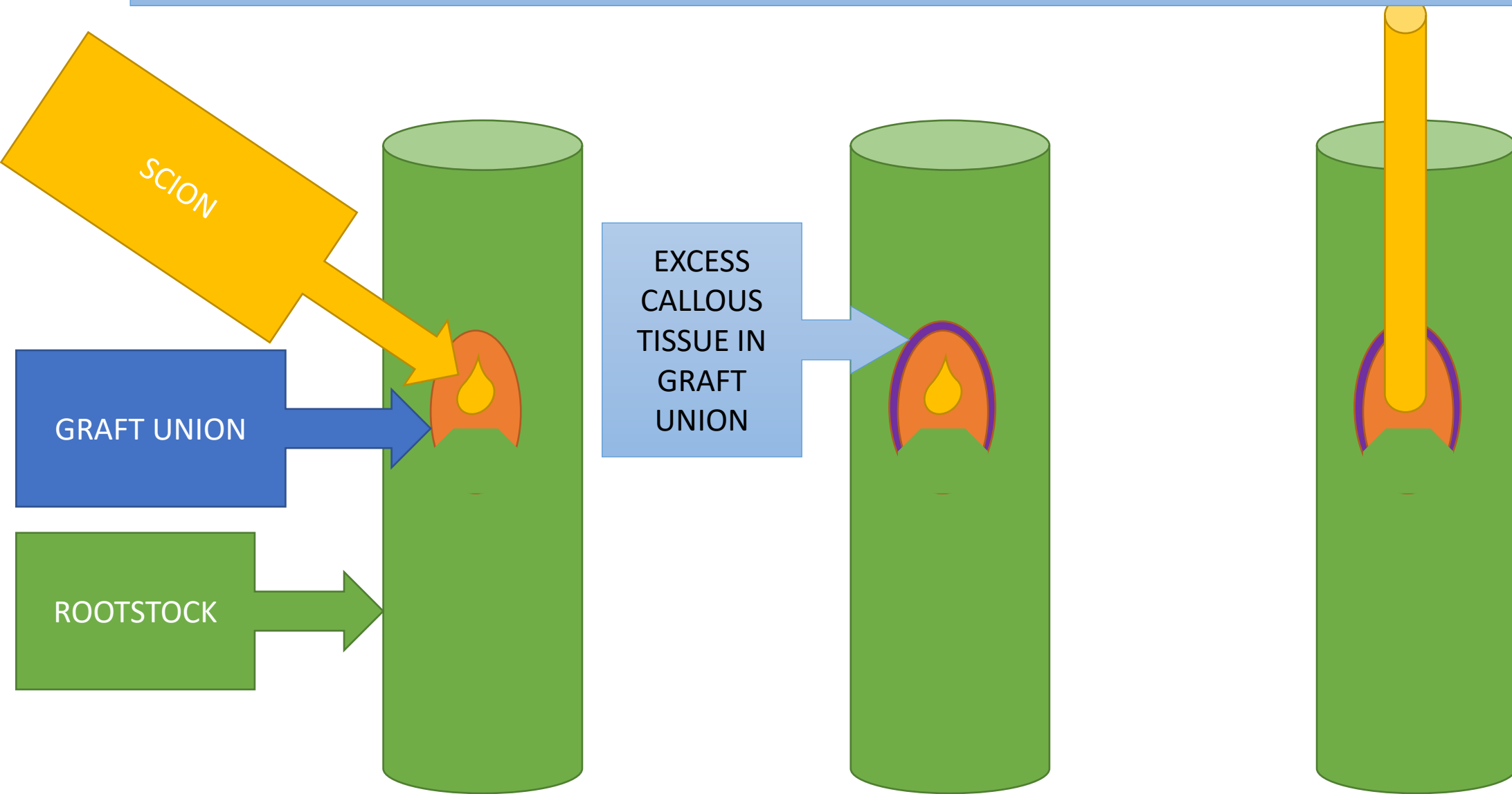
As the tree grows tissues from the scion and rootstock develop in sync

Improper graft union as seen in some Gala trees with excessive callous

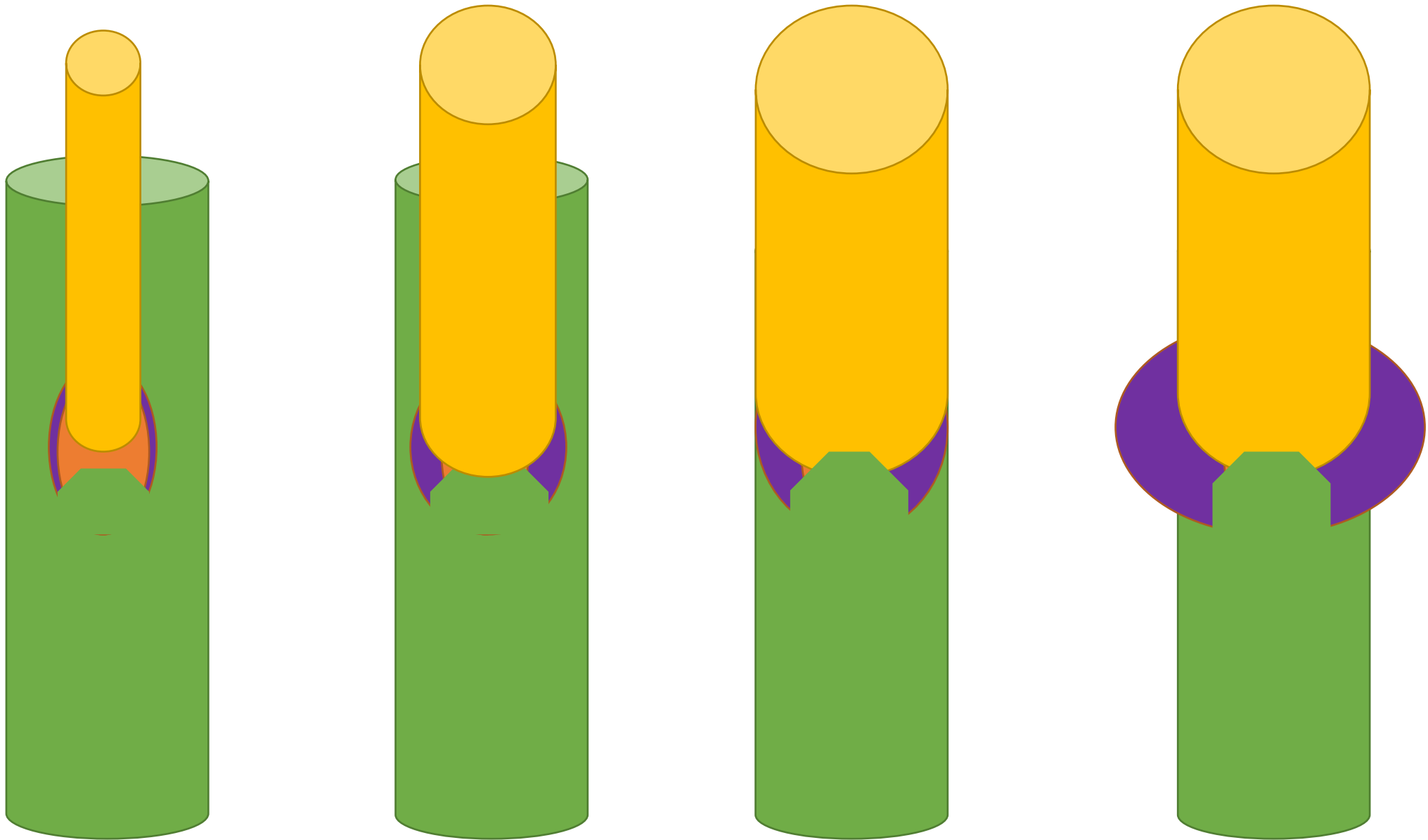


As the tree grows tissues from the scion and rootstock develop out of sync causing a restriction between the scion and rootstock preventing good growth

Similar formation with excessive callous around chip buds

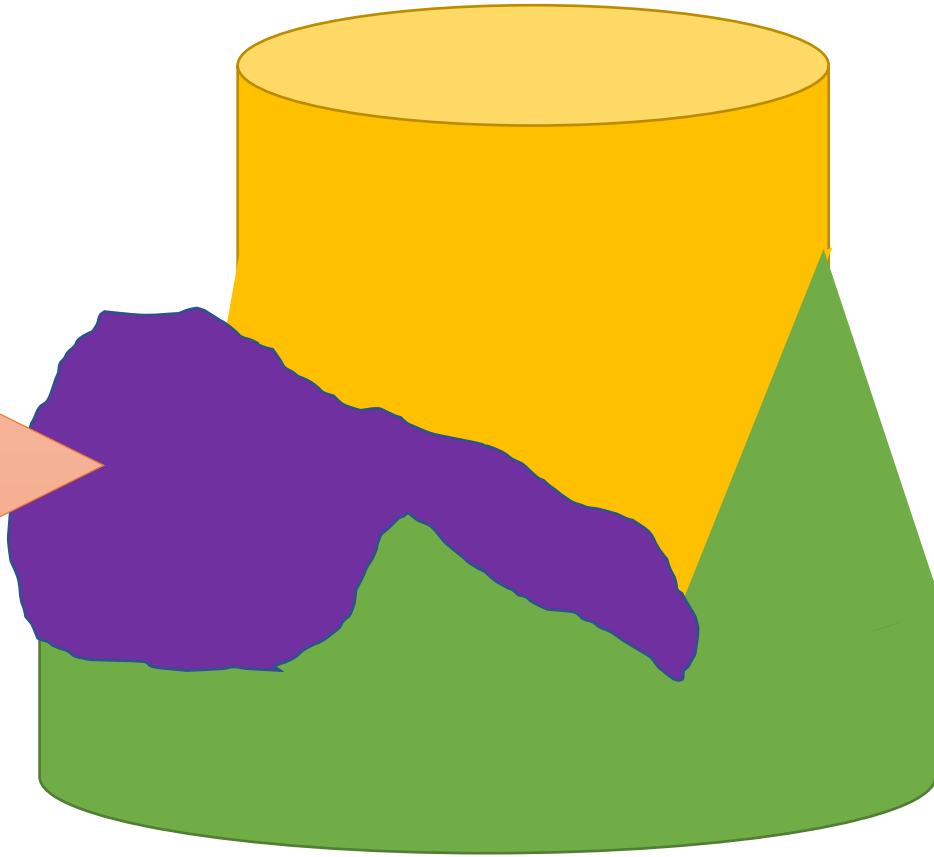


Similar formation with excessive callous around chip buds

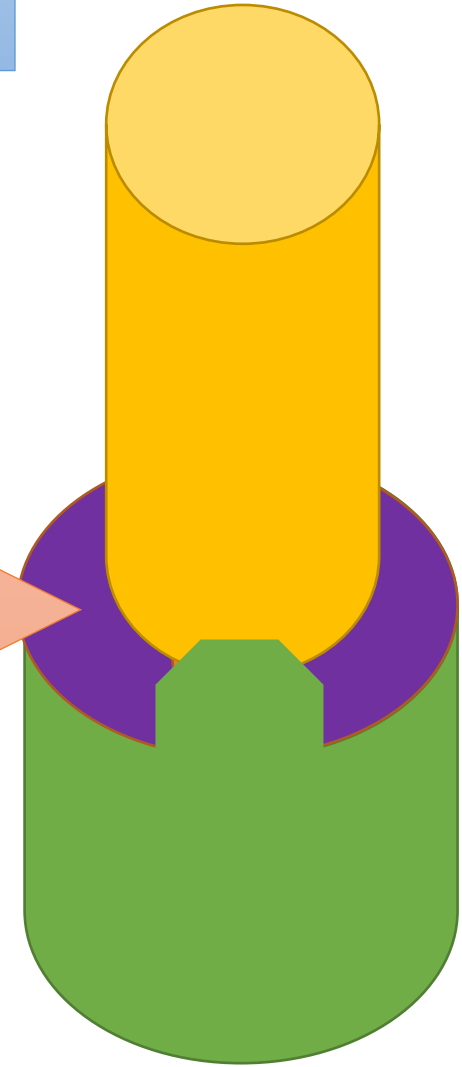


Similar outcome for chip and whip and tongue grafts with excessive callous around chip buds

Disruption of
phloem
feeding of
root system,
unorganized
tissues,
proto-endo-
burr-knot
formation,
broken bark



Disruption of
phloem
feeding of
root system,
unorganized
tissues,
proto-endo-
burr-knot
formation,
broken bark



Working hypotheses given the Brazil Data

- The problem occurs with certain varieties that are prone to making burr knots on their own.
- **The problem involves callous tissue (perhaps a chimera of two genotypes – rootstock and scion) of which one is prone to generating burr knots, however it may be that predominantly G.213 callous tissue is changed genetically (similar to changes that lead to totipotent cells – major tissue gene expression reprogramming).**
- Internal burr knots are the cause of unorganized tissue proliferation (root tips producing cytokinin).
- Internal burr knots are stimulated in wet environments (irrigation, protective tubes).
- Unorganized tissue proliferation interrupts proper communication and feeding of root system as photosynthate is accumulated in the form of amylose – good feeding ground for insects and fungi.
- Insects, fungi, and animals (voles and rabbits) attack weakened tissues below the graft union and are the final causes of collapse of the trees.
- **Constitutive hormonal balance of G.213 collides with hormonal balance of scion varieties that are prone to making burr knots. It is likely that G.213 interdicts the normal translocation of auxins from the scion which are then accumulated at the boundary of the graft union. The callous tissue (undifferentiated) receives a very strong signal to produce root initiations instead of normal vascular tissue. We are in the process of testing this hypothesis.**

Update from March 2023 Brazil visit

- Visited additional nurseries that were growing G.213.
- Collected DNA and other samples.
- Considered possible solutions.
- Eliminated the possible involvement of micro-propagation from the issue (watching plants from stool bed). The side-by-side picture(right) shows with no doubt the source of the problematic tissue.



Update from March 2023 Brazil visit - RACIP

- The initial RACIP trial was planted with stool bed derived liners featuring Gala and Fuji.
- No problems found in the Fuji part of the trial. Some problem graft unions (low percentage of trees) found with Gala.
- Most of the burr knot formations were found above the graft union.
- Some of the proto-endo-burr-knots were found in the graft union.
- DNA test at the graft union confirmed presence of Gala DNA suggesting that the mixed Gala/G.213 callous tissue as being the source of the problem. However, some DNA samples indicated that these formations were being produced in callous tissue almost exclusively made of G.213.



Update from March 2023 Brazil visit – Leandro Nursery

- We observed graft unions at different stages of development and with both Fuji and Gala scions.
- The quality of the graft unions is the source of the mixed callous tissue that seems to be problematic.
- We can see the progression of callous tissue developing between the large gaps of the graft and some proliferation already happening.
- DNA test of the callous tissue at the graft union of Fuji trees confirmed presence of ONLY Fuji DNA. DNA test of callous tissue of Gala trees showed mixed Gala/G.213 callous tissue.



Update from March 2023 Brazil visit – Leandro Systems Trial Orchard

- This systems trial features Fuji and Gala on different rootstocks.
- No problems found in the Fuji part of the trial. Problem graft unions (high percentage of trees) found with Gala.
- Most of the burr knot formations were found in the graft union. You can see the proliferation of tissue at the union matches the amount and type of tissue that forms in young trees.
- All of the proto-endo-burr-knots were found in the callous derived tissue at the graft union.
- DNA test at the graft union confirmed presence of Gala DNA confirming that the mixed Gala/G.213 callous tissue as being the source of the problem. The proto-endo-burr-knots are originating from Gala tissue.



Update from March 2023 Brazil visit - Nursery

- A nursery near Lages.
- Some of the worst graft unions with Gala – they are healed and the trees are growing very well.
However, this type of grafting practice produces the most callous tissue.



Update from March 2023 Brazil visit – Rufato Lab

- We took some samples from the Gala/G.213 systems trial into the lab and dissected them.
- Evidence of starch containing tissue (usually associated with root tissue) found when stained with iodide solution.
- Proto-endo-burr-knot formations were associated with graft union callous.
- DNA test at the graft union confirmed presence of Gala DNA confirming that the mixed Gala/G.213 callous tissue as being the source of the problem.
- Most Brazilian nurseries practice live whip and tongue grafting (mid summer) which induces the formation of a lot of callous tissue.



Problem is exacerbated by the use of rodent protection barriers

- Rodent protection barriers seem to cause fungal/bacterial/insect pressures on tissue that derives from these proto-burr-knots.
- Need to use larger barriers that avoid the accumulation of wet debris between the tree shank and the plastic.





More rodent protection barriers

Update from March 2023 Brazil – Sao Joachim Trial

Beautiful production and no problems with Fuji



Update from March 2023 Brazil – Sao Joachim Trial

No issues detected with Gala.
Different nursery and nursery stock.
Gala burr knots are above the graft union.



New York, California and Washington

- Some of the oldest trials in the U.S. are in New York 2015 (Golden and SnapDragon) and Washington (with Cosmic Crisp).
- More recent trials in California with Fuji, Honeycrisp and Gala derived from micro-propagated and stool bed liners.
- All reveal some interesting things that substantiate earlier observations



Difference between WA-38 in WA and Fragsburg



WA-38 on G.213 in Fragsburg. Variability in formations. Wet environment with trunk protectors. The syndrome is likely exacerbated by the wet environment.



WA-38 on G.213 in George, WA. No presence of syndrome in any of the trees. Planted in 2017.

Organic Trial, Cuyama Orchards, California



2015 Golden Trial Williamson, NY



These trees were chip budded in August 2013 on liners that were harvested from a stool bed in Geneva in November 2012. Finished trees were planted in 2015. There is a small burr knot emerging from the graft union and a bulge below, but trees are very healthy and productive. Golden Delicious does not produce burr knots – according to experts I have inquired with. The presence of the burr-knot and the bulge below the graft union support the hypothesis of auxin buildup at the graft union, perhaps because it is not very well transported into the roots by G.213 tissue (we are in the process of studying this).

Additional pictures and data from Italy (2023)

Trees produced by August chip budding, performing very well with no problems.



Analysis of results

- All observations indicate the involvement of callous tissue in combination with certain cultivars that are prone to burr-knot formation.
- Good graft union with minimal callous and good vascular system is associated with lack of problems.
- According to an Italian nursery that has been working with G.213 for several years and practices mostly chip budding (August), the problematic trees (about 2% of the crop) can be culled before sold. Compared to the type of grafting (Spring-Summer live trees) in Brazil which produces a lot of callous tissue and therefore sets up the trees for the formation of this problem.
- Dormant budding or grafting seems to reduce or eliminate the problem completely.
- Problem is exacerbated by plastic rodent protection or excessively humid environments.

Nearly perfect row of G.213 with Innored scion variety in a well managed orchard



Great performance of G.213 in Brazil

- On the right is a row of Gala trees grafted on G.213 at the Fisher establishment near Fraiburgo Brazil. Trees are healthy and are producing lots of apples compared to the row on the left (Marubakaido rootstock with M.9 interstem)
- Still, the type of grafting in Brazil seems to generate a lot of callous tissue and differences among nurseries (type of budding/grafting, timing of year, dormant vs actively growing) can explain the presence/absence of this problem in orchards planted in different areas.



What next?

- Visited 30+ commercial plantings and rootstock trials in these trips.
- The performance of the G.213 rootstock unaffected by this syndrome is outstanding compared to traditional rootstocks.
- Recommended increased monitoring and killing of borer insects and increased use of fungicides on the rootstock shanks on affected trees.
- Recommend dormant budding/grafting which reduces the formation of callous tissue.
- Recommend planting graft union closer to ground in some Brazilian plantings.
- Recommend to delay nursery grafting and planting of micro-propagated G.213 rootstocks until they have gone through a dormancy period.
- Recommend testing the removing of root tips in the endo-burr-knot formations and painting with copper compounds or other compounds that cauterize and protect the wounds.
- Recommend testing NAA paint in nursery and young trees to suppress burr knot formation at the graft union
- Replication of this phenomenon in Geneva with the same scion varieties and document/study this newly discovered interaction.

