Biology and Assessment of Callus and Woundwood

By Christopher J. Luley

Objectives

- Explain the circumstances in which callus and woundwood are formed
- List the environmental and physical conditions that affect growth of callus and woundwood
- Understand the implications of callus or woundwood on tree risk assessment

CEUs for this article apply to Certified Arborist, Utility Specialist, Municipal Specialist, Tree Worker Climber/Aerial Lift Specialist, and the BCMA science category.

Tree response to wounding has attracted much attention from pathologists and botanists for the past two hundred years (Stobbe et al. 2002), and man has been attempting to treat tree wounds for over 4,000 years (Neely 1979). The reaction of trees to wounding is also a topic of importance to arborists who regularly wound trees by pruning, and are also called upon to evaluate tree response to wounding in risk and Plant Health Care assessments (Figure 1).

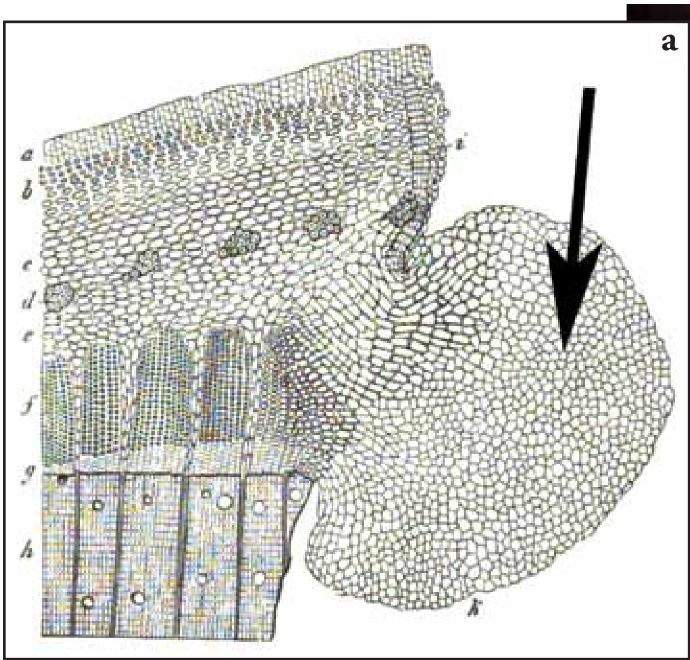
Historically, wound response has been divided into wound closure (new growth formed after the wounding event and discussed in this article) and compartmentalization (various responses of preexisting tissues) (Shigo 1984). Two terms have dominated the discussion of wound closure, *callus* and *woundwood*. These terms have been a source of confusion for both scientists and arborists. This article will review the biology of callus and woundwood formation, and demonstrate how this knowledge can provide diagnostic information about overall tree health, reaction to pathogens and insect pests, tree stability, and forensics.

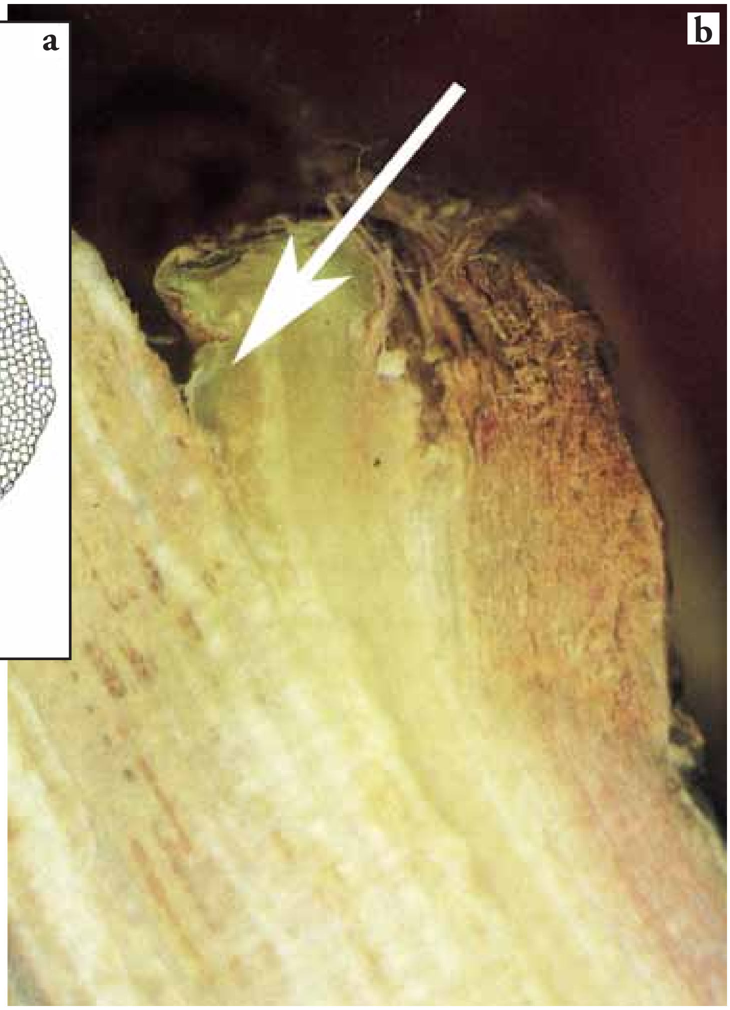

a


b

SHIGO (1989)

Figure 1. Arborists wound trees by pruning, and evaluation of woundwood development is an indication of whether proper pruning cuts were made on the branch. Figure 1a (above) shows a complete ring of woundwood around a red maple (*Acer rubrum*) pruning wound, indicating a proper pruning cut. Figure 1b (right) shows dieback of tissues around the top and bottom of a pruning wound from an improper pruning cut.





SHIGO (1994)

Figure 2. Drawing (above) of callus (arrow) developing from the bark and cambial zone from an oak (*Quercus*) branch (Hartig 1894). Figure 2b (right) shows callus forming along a root tear. Callus (arrow) may have chlorophyll as seen in this image.

Callus v. Woundwood

Unfortunately, the term callus has been used interchangeably with the term woundwood almost since the terms were coined.

According to Wikipedia, *callus* was first used in the early 18th century to designate cell growth in elms after wounding. Küster (1913) later identified callus as "homogeneous, parenchymatic" and "very thin walled, undifferentiated cells" (Figure 2) that generally lack lignin (Shigo 1989). Ikeuchi et al. (2013) indicated the term is now used more broadly in botany to describe disorganized cell masses, citing several references that suggest varying levels of genetic and organ differentiation can occur in a callus. Fink's (1999) authoritative discussion of callus represented the historical, more conservative definition; he referred to it as undifferentiated parenchymatic proliferations frequently of mixed origin but finally having a homogenous appearance. So, even today, scientists still vary on their definition of callus.

In contrast, *woundwood* is highly organized wood with lignin (Shigo 1989). The term was coined by de Vries (Hartig 1894) when he observed that wood formed after wounding had shorter than normal cells with a scarcity of vessels and medullary rays (Hartig 1894) (Figure 3). Küster (1913) broadened the term woundwood to include tissues formed after wounding that appeared similar to wood (Figure 4).

So, why the confusion? It likely stems from several facts. One is that callus formation initially precedes woundwood but soon (within months) differentiates to produce vascular cambium, which then produces woundwood. So callus is quickly obscured by this developing woundwood and is seldom observable—except in the initial weeks or months after its formation. Therefore, the tissue visible to arborists in the years after wounding is woundwood, while one of the tissues it is initially generated from is callus.

Despite the clear anatomical and functional differences between woundwood and callus, many arborists, older scientific publications, and even some recent texts still use the terms interchangeably. A closer look at the biology of callus tissue and woundwood formation will help explain why confusion persists in the scientific and arboricultural world, and why proper use of these terms is important.

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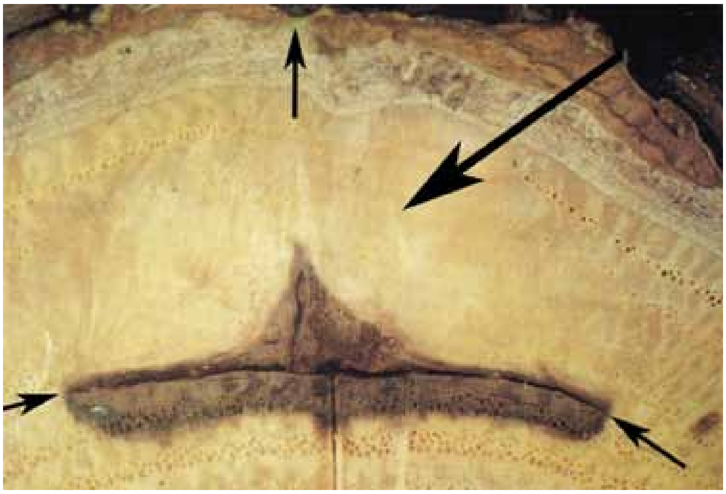

Figure 3. Woundwood (large arrow) has sealed a small wound (lower, smaller arrows) on an oak (*Quercus*). Note how the woundwood mostly lacks vessels at this stage and how bark has rejoined to completely seal the wound (upper, small arrow).

Callus and Woundwood Biology

Woundwood develops from callus or from uninjured vascular cambium at the margin of injuries that have damaged or exposed the phloem, vascular cambium, or sapwood (Fink 1999). Shallow wounds that only damage the outer bark do not stimulate the production of woundwood. Bark reacts to wounding in its own distinct way by forming wound or necrophylatic periderm (see Hudler 1984 for a good discussion of this process). However, cells in the phloem or inner bark can contribute to the formation of callus and woundwood (Figure 2).

Callus

Callus development can be detected within weeks in actively growing trees, usually after cells on the edge of the wound die. Callus is produced by enlargement (hypertrophy) or increased division (hyperplasia) of cells adjacent to the edge of cell dieback. Living cells without

Figure 4. With time, woundwood (arrow) develops the same as normal sapwood with normal bark and cambium. Counting annual rings in woundwood is one method used to determine when a wound occurred in a tree. This aging method requires finding the barrier zone formed in the oldest annual ring present when the wound occurred and counting annual rings that formed after in woundwood or the sapwood. This process is generally more complex than presently stated; however, to detail it extensively is beyond the scope of this article.

secondary cell walls can produce callus, including sapwood ray parenchyma, vascular cambium, and parenchyma cells in the bark (Küster 1913; Neely 1979).

If patches of bark are removed that leave behind cambium and undifferentiated xylem, callus can form directly on the surface of the wound. Because of this, bark and cambium can re-form and woundwood can develop even when large patches of bark are removed (Figure 5). Stobbe et al. (2002) showed for *Tilia* that callus on the surface of these injuries had formed wound periderm (bark) before the formation of vascular cambium.

Cells from the vascular cambium usually contribute significantly to callus, but callus can also form from living ray cells in the absence of living vascular cambium (Noel 1968). Regardless of origin of the callus, new vascular cambium can form from it and produce woundwood xylem and phloem. After vascular cambium formation, the callus cells no longer divide but lignify while retaining their isodiametric shape. The new vascular cambium continues to divide and covers the callus from which it was derived (Kevin T. Smith, USDA Forest Service, pers. comm.).

Figure 5. Woundwood that formed on the face of a bark flap that had been removed from a white ash (*Fraxinus americana*). The woundwood is generated from callus that formed from vascular cambium and undifferentiated xylem left when the bark was removed. Also note the separate woundwood response on the edge of the wound.

Figure 6. Callus can differentiate to form adventitious shoots and other plant organs. These shoots are growing out of woundwood that developed on a silver maple (*Acer saccharinum*).

Callus growth itself can seal small wounds, or form extensively over larger surfaces when larger patches of bark are removed, but it is usually covered over by woundwood within the first growing season. The rate of callus formation varies due to several host and environmental factors but is usually fastest on young or fast-growing trees. Callus and woundwood growth varies considerably amongst tree species (Neely 1979; Marshall 1931) and has been related to insect pest resistance, such as the red oak borer in red oak (Fierke and Stephen 2008).

Callus can be formed by most organs of trees, including stems, roots (Figure 2b), leaves, and fruit (Küster 1913). Once formed, callus is *totipotent*, meaning it can be induced to form the entire plant (Ikeuchi et al. 2013) or individual organs, such as shoots (Figure 6), roots (Figure 7), or buds. It has long been known as a result of in vitro culturing that varying ratios of the plant hormones auxin and cytokinin influence what type of organs develop from callus.

Vascular Cambium and Woundwood

Woundwood forms from vascular cambium that differentiates from callus or can form directly from uninjured vascular cambium. Depending on the time of year, callus can develop and vascular cambium can differentiate from it within a few weeks after wounding (Neely 1979; Oven and Torelli 1999; Copini et al. 2014a). Once the vascular cambium is formed, woundwood with xylem (wood) and phloem (inner bark) can grow to start to seal larger wounds not sealed initially by callus.

Vascular cambium produced by callus usually becomes continuous with pre-existing vascular cambium after its formation (Küster 1913). The vascular cambium on both sides of a wound will also join and become continuous if callus and woundwood quickly seal or cover over a wound (Figure 3). Shigo (1989) pointed out that natural cracks may form where woundwood and callus initially join and seal a wound, and this can be the source of future seams or cracks that never completely seal.

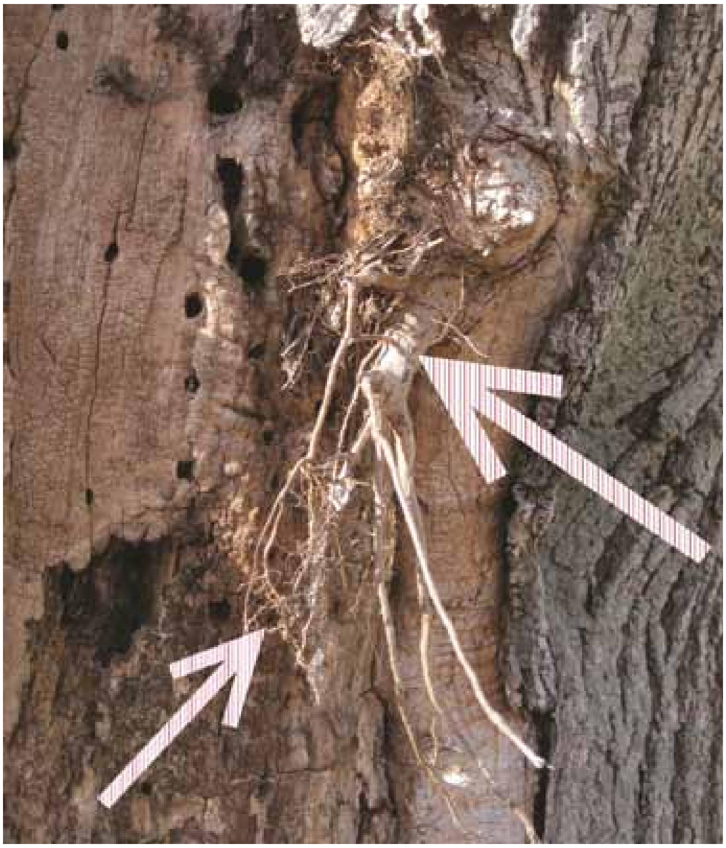

Figure 7. Aerial roots (arrows), shown here on Norway maple (*Acer platanoides*), can form from the differentiation of callus after wounding.

SHIGO (1989)

Figure 8. Ram's horns formed by the inward turning of woundwood. They prevent any chance of the wound ever sealing.

Normal bark, with its own bark cambium, can form from callus, or it can extend from existing bark on the edges of the wound. Also, woundwood has no definite or predetermined form but commonly takes the shape of the wound and callus that formed along the wound (Küster 1913) (Figure 8; Figure 9).

As many studies have shown, woundwood that forms initially is anatomically different than normal xylem sapwood. In conifers, woundwood typically has increased density of resin canals (Oven and Torelli 1999). In deciduous trees, cells may have increased lignin content, thicker secondary cell walls, and cells are often shorter than in normal wood (Küster 1913; Smith 1980; Frankenstein and Schmidt 2006). Vek et al. (2013) recently showed that woundwood in beech (*Fagus* spp.) also contains extractives that are inhibitory to some fungi. With time, woundwood appears essentially the same as normal sapwood (Figure 4).

Growth of callus and woundwood is faster towards the open face of the wound, presumably because of the absence of pressure from bark and other tissues (Hartig 1894). This effectively helps seal the wound more quickly. Callus and woundwood can seal small wounds [0.5 inches (12.7 mm) or less] in one growing season on fast-growing trees of some species (Neely 1979). Once the wound is sealed or covered by woundwood, further discoloration of the sapwood is slowed (Sinclair and Lyons 2005) and progression of decay may be stopped or inhibited (Fink 1999). Wounds not sealed by woundwood over several growing seasons almost always result in decay. For example, stem wounds larger than one square foot (900 cm^2) are twice as likely to become decayed as smaller wounds (Greifenhagen and Hopkin 2000).

Ram's horns are often found on large wounds if the woundwood curls into an opening or cavity formed by the wound or subsequent decay, or if thick bark forms on opposing ribs of woundwood that prevent their confluence (Figure 8). Ram's horns usually prevent any chance of the wound eventually sealing (Shigo 1989) (Figure 8).

Research by Kane and Ryan (2003) showed some red maples have woundwood that is tougher and denser than normal sapwood. This appears to support common knowledge of the elevated strength and toughness of woundwood, and its use in hand tools where these characteristics are advantageous.

Timing of Callus and Woundwood Formation

Callus and subsequent woundwood development closely track cambial activity in trees. Numerous research articles

Figure 9. Woundwood usually takes the shape of the original wound that caused the injury.

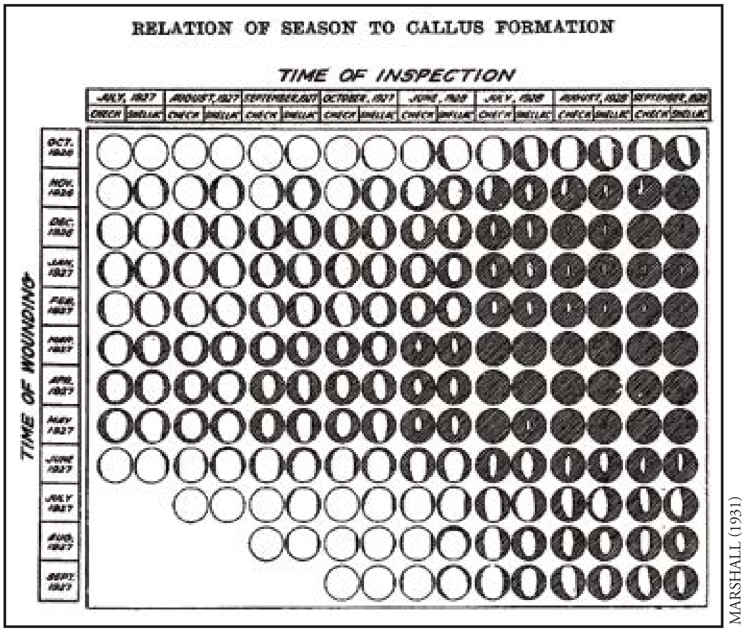

Figure 10. This figure shows wound closure from wounds made at different times of the year and treated with a wound dressing. Wound closure is fastest on wounds with or without the shellac treatment made just before growth starts or when the tree is actively growing.

have reported that wounds made after growth ceases, or made during the winter, will not form callus and woundwood until the next spring. These wounds will also seal more slowly once growth resumes (Figure 9). Dieback around wounds is also greater when wounds occur in autumn (Neely 1988). Conversely, callus and woundwood formation is fastest after wounding, and the amount of woundwood produced is greatest (and wound closure is therefore fastest) on wounds made shortly before or early in the season of active tree growth. Recent research has also shown that callus and woundwood can form even when trees are dormant, if temperatures are moderate (Copini et al. 2014a).

The Heal v. the Seal

Shigo (1989) taught that wounds do not heal in the sense of restoring or replacing damaged tissues in the same spatial position. Rather, trees replace lost tissues and their function by sealing or closing over the wound surface. Both callus and woundwood can seal wounds, but woundwood is the tissue that seals larger wounds in trees and returns the function to the stem.

It is generally accepted that wound dressings are not needed and generally do not increase wound sealing or decrease decay, possibly because of their inhibitory effect on callus and woundwood formation. However, some studies have shown that Shellac® and Lac Balsam® can increase initial wound closure on some species depending on the time of the year they are applied (Marshall 1931; Hudler and Jensen-Tracy 2002). McDougall and Blanchette (1996) showed that polyethylene plastic wrap increased callus and reduced dieback around wounds when applied right after wounding on certain tree species. This effect may have been due to the enhancement of conditions, such as high humidity, which apparently foster callus development along the edge of wounds (Hartig 1894).

Woundwood Assessment

Woundwood is considered a type of response growth that requires evaluation in tree risk assessment (Smiley et al. 2011). Close observation of the presence or absence of woundwood, the amount and rate of formation, and its age can provide valuable information on when wounds occurred, tree health, and infection and resistance to pests.

Presence or absence of woundwood formation is a key observation. Lack of woundwood formation after a full growing season or more can be due to several factors, some of which can have implications for tree health and the capacity of trees to contain decay or other pests. Neely (1988) indicated callus growth is regulated by basipetal (crown to root) flow of carbohydrates and growth

Figure 11. The general absence of woundwood development on this old sugar maple (*Acer saccharum*) wound is an indicator that the tree may lack the vitality to respond to the wound, thus increasing the likelihood other defense mechanisms that contain decay are also compromised.

Figure 12. The absence of woundwood around the dead tissues on this beech (*Fagus* spp.) infected with *Phytophthora* sp. and *Kretzschmaria deusta* indicate these pathogens are actively killing bark and cambium.

regulators. Callus and woundwood may not develop, or develop slowly, if radial growth is slow in a particular area (Neely 1988). Physical restrictions, such as included bark, may also inhibit callus and woundwood formation after a branch is removed (Shigo 1989). Shigo (1989) also showed how the evaluation of woundwood around pruning wounds indicates whether a branch was pruned properly (Figure 1).

Absence of woundwood formation can mean the tree did not have adequate vitality (measured in this case as available growth resources) to support its growth (Figure 11), or the tissues were damaged beyond response before, during, or after wounding. Absence of woundwood formation may also be due to the presence of pathogens (usually fungal) that are killing woundwood or the tissues involved in woundwood response (Figure 12). In both cases, absence of woundwood formation indicates a tree's defense mechanisms are likely compromised, and could indicate decay or other pathogens are not being slowed by active tree defense responses.

Forensics

As woundwood grows, it produces annual rings the same as annual growth increments in normal wood. On some species, annual increments of bark and woundwood growth can be seen on the surface of the woundwood (Figure 13), providing a general indication of the age and rate of woundwood formation. Species differ in their rate of woundwood production (Marshall 1931; Neely 1979), and so species differences should be accounted for when assessing woundwood production.

Internally, woundwood forms annual wood growth increments as in normal xylem growth. Counting these annual increments is a reliable method to age wounds, as well as cavities or decay columns that formed after a wound if the annual ring present at the time of the wound can be identified (Figure 4). This aging method requires finding the barrier zone formed in the oldest annual ring present when the wound occurred and counting annual rings that formed after in woundwood or the sapwood. The year and time of year that insect borer pest damage occurred have been dated using this method (Copini et al 2014b).

Woundwood formation along a crack can also provide evidence for the presence and age of cracks between codominant stems or branch unions (Figure 14), or in wood before or after a stem failure. Assessment of the woundwood can therefore provide evidence of the location, age, and length of the crack.

Summary

Callus is formed first in response to wounding by cells in the bark, cambium, and sapwood. Shortly after its formation, it differentiates to form woundwood. Initially, woundwood is anatomically different than normal wood.

Figure 13. Annual increments of growth (arrow) are sometimes visible on the surface of woundwood, giving an indication of the rate of its formation. Woundwood was developing relatively rapidly on this Norway maple (*Acer platanoides*) wound.

Figure 14. Evaluation of woundwood formation can provide forensic evidence about tree failures. Woundwood was present when the branch failed, forming along the edge of where the branch separated from the trunk prior to failing. The arrows show where woundwood stopped at the base of the crack.

The rate of callus and woundwood formation is influenced by species, tree health, and the time of year a wound occurs. Callus and woundwood formation are fastest when wounds are made just before the growing season starts or when cambium is actively growing. Once woundwood or callus seals a wound, discoloration and decay of sapwood are slowed or may not develop further. Evaluation of callus and woundwood formation can provide evidence of when the wound occurred, tree health, and the response of a tree to pest attack.

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1. Woundwood may have increased resistance to decay compared to normal wood.
   a. True
   b. False
2. Wound dressings are known to
   a. promote woundwood and stop decay
   b. inhibit woundwood and have little impact on decay
   c. enhance tree response to decay and borers
   d. promote tree defense response to pests and protect wounds
3. Callus tissue develops around wounds
   a. shortly after wounding
   b. before woundwood
   c. is usually obscured by woundwood
   d. all of the above
4. Dieback around wounds and delayed production of woundwood is most noticeable when wounds are made in
   a. spring
   b. summer
   c. autumn
   d. winter
5. Callus develops from
   a. vascular anomalies and bark parenchyma
   b. meristematic points
   c. hyperplasia and hypertrophy of cells adjacent to wounds
   d. meiosis and mitosis
6. Woundwood is considered a type of response growth.
   a. True
   b. False
7. Callus or woundwood growth are *not* inhibited by
   a. proper pruning cuts
   b. cold weather
   c. included bark
   d. restricted flow of carbohydrates
8. The terms callus and woundwood
   a. indicate the absence of meristematic tissue
   b. refer to production of chlorophyll
   c. can be used interchangeably
   d. refer to anatomically different tissues
9. Presence or absence of woundwood is an indication of
   a. wound size
   b. wound type
   c. wound response
   d. wound healing
10. Woundwood develops from
    a. callus or uninjured vascular cambium
    b. novel response of bark sieve tubes
    c. ray parenchyma and outer bark parenchyma
    d. rapid division of meristematic points
11. Woundwood and callus sealing of a wound
    a. can be a source of future cracks
    b. traps decay organisms in the tree
    c. prevents insect damage
    d. all of the above
12. Ram's horns are formed by
    a. the inward turning of woundwood
    b. tangential formation of callus
    c. callus inhibiting inward spread of decay
    d. delayed tree response to wounding

13. What organs develop from callus is largely influenced by the varying ratios of
    a. auxin and cytokinin
    b. abscisic acid and auxin
    c. woundwood to callus
    d. all of the above

14. Which of the following is *not* a reason why woundwood evaluation could be part of a tree risk assessment?
    a. indicates presence of infection
    b. formation of woundwood is indicative of tree health
    c. may indicate resistance to pests
    d. response growth proves prior site changes

15. Growth rings in woundwood indicate
    a. the presence of decay
    b. decay severity
    c. the age of the wood
    d. future wound response

16. The two main categories of wound response are
    a. compartmentalization and dieback
    b. wound closure and compartmentalization
    c. callus tissue and wound closure
    d. wound closure and woundwood

17. Anatomically, when compared to normal wood, woundwood
    a. is the same
    b. has increased lignin
    c. has increased suberin and lignin
    d. changes as it is formed but is eventually the same

18. Callus is *totipotent*, meaning it can
    a. form the entire plant, including stems, roots, and leaves
    b. differentiate to form chlorophyll
    c. develop new heartwood
    d. all of the above

19. Woundwood is formed
    a. equally at all times of the year
    b. mostly in the dormant season
    c. fastest when the tree is actively growing
    d. intermittently, depending on photosynthate availability

20. Bark parenchyma remaining after wounding can contribute to callus and woundwood formation.
    a. True
    b. False