



Outreach

Warnell School of Forestry & Natural Resources

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Black Walnut Allelopathy: Tree Chemical Warfare

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The biological system of a tree internally manages the economics of essential elements, water, light, and metabolites to control health and growth. Externally, a tree must adjust to the presence of other organisms and to the constraints of its site. Problems of surviving and thriving are associated with capture and control of ecologically viable space, competition for essential inputs, and chemical coadaptation to the presence of other organisms and to site attributes.

The chemical coadaptation to other organisms and site is termed “allelopathy.” Allelopathy is the use of many different chemicals produced by many trees to interfere with the growth and development of surrounding plants and microorganisms. This interference impacts essential resource quality and quantity available on a site.

Poisoned!

One of the oldest known and most noticeable allelopathic effects in trees occurs in walnut (*Juglans* spp.) associations. As early as 77 AD, the *Juglans* genera was cited by a Roman naturalist as having a poisonous effect on other plants. Down through the years, many people have used this toxic characteristic of walnut to poison fish, treat human and animal diseases, keep broad-leaved weeds out of lawns, and control garden plants. Dead tomatoes and other garden vegetables, sickly azaleas, and stressed broad-leaved plants have all been a direct result of walnut poisoning.

Chemical Stew

Not every tree chemical has a significant ecological / allelopathic impact. For example, more than 35 phenolic compounds have been identified in walnuts, and more than 12 compounds just in the green fruit husk. Many of these materials have been cited as expressing some level of allelopathic symptoms in other plants and microorganisms. These compounds tend to peak in concentration early in the growing season after full leaf expansion because of their role in new tissue protection. Several of the identified phenolic compounds of walnuts have been shown to generate significant allelopathic impacts on surrounding plants. By far the chemical with the largest concentration, and one of the most toxic, usually present at >20 times more than all the other allelopathic materials in walnut, is juglone.

A number of these phenolic compounds in walnut can act as antioxidants and are antimicrobial. English walnut green husks, for example, have been used in health promoting tonics due to varied phenolic contents. Juglone has also been considered as a food additive, wood preservative, and antifungal / antibacterial agent. Juglone can also be found in other tree species conveying protective properties, such as in teak bark (*Tectona grandis*).

The Black Walnut

Black walnut (*Juglans nigra*) of Eastern North America was first mentioned as having an inhibitory effect on other vegetation by Hoy and Stickney in 1881. Since that time, numerous authors have reported inhibition of plant growth in close association with black walnut trees.

The active agent inhibiting growth of other plants was suggested by Massey in 1925, and then confirmed by Davis in 1928. The active allelopathic agent was juglone (5-hydroxy-1,4-naphthoquinone). Figure 1. The effect of juglone on other plant tissues is to inhibit hydrogen ion transfer essential to energy production in respiration, as well as inhibiting two different oxidation reactions. At low concentrations (i.e. $<3 \mu\text{M}$), juglone can disrupt the electron transport system. At higher concentrations, juglone increasingly inhibits oxidative phosphorylation.

Juglone is so toxic only minute amounts can sicken, sedate, or kill people and animals. The concentration difference for juglone between that needed for sedation, and that causing death, is small. Juglone disrupts oxygen and food use in both plants and animals -- a respiration poison. Juglone is similar to cyanide in its effect on people, animals, and plants.

Protection

Juglone precursors are produced and juglone is generated by a tree for protection from leaf, root and stem pests, like insects, diseases, nematodes, and grazing animals, as well as interfering with surrounding plants / weeds. The immediate precursors of juglone are found in high concentrations within buds, flowers, fruit, and in phloem. Figure 2. Juglone precursors appear to be translocated from older tissue to younger tissue over time. Under oxidative conditions outside of living cells, and during and after injury, juglone is formed.

Manufacturing

The metabolic "net" of juglone synthesis is given in Figure 3. The chemical compounds involved in juglone formation and their equilibrium states, suggest measurement of juglone concentration in tissues alone may not be an accurate method for gauging the ecological significance of juglone to a particular site. Instead, the total amount of potentially active material must be assessed. Because juglone precursors move toward juglone in an oxidative environment, determination of juglone availability in walnut tissue under oxidative conditions provide a more accurate and meaningful ecological input for this allelopathic agent.

Juglone is found in only minute amounts within walnut tissues because of its toxicity. Juglone exists within a tree in a non-toxic form called hydrojuglone. Juglone is held in its reduced forms, principally (alpha-hydrojuglone = 1,4,5-trihydroxynaphthalene), and in a bound state with glucose inside living tree cells. Hydrojuglone is colorless and generally nontoxic, but is immediately converted to juglone by oxidation. Upon continual contact with oxidative conditions, or tissue drying, juglone is tied up and decomposed.

Toxic Color

Hydrojuglone manufacture and storage is how walnuts can hold juglone protection without damage. Hydrojuglone is kept in tree buds, flowers, fruit husks, leaves, roots, and in the secondary

cortex just under the periderm. If any tree cells are damaged, eaten, cut, or injured, hydrojuglone is immediately oxidized into its toxic form (juglone). Insects, diseases, and mechanical injury can all cause juglone generation.

Juglone is dark brownish-red in color. Hydrojuglone is clear. Oxidation converts hydrojuglone to juglone. A demonstration of this change in color caused by juglone can be seen when cutting open a green walnut husk. The green nut husk will quickly turn brown when exposed to air. The color change is caused by the clear, non-toxic hydrojuglone being quickly converted into the toxic, dark brown juglone in the presence of oxygen.

Only by exposure to air does juglone form. In the past people have made use of this color change. Boiled walnut husks have been used for ages to dye cloth and basket making materials to a dull brown color. It has also been used to dull the surface of shiny iron (a form of bluing). Extracting nut meats can leave hands and gloves stained from juglone formation.

Potential

The literature concerning extraction and quantification of juglone frequently fails to differentiate between juglone and its immediate precursors. The term “juglone potential” should be used to define the total concentration of juglone generated under an oxidative environment. The juglone potential of a given tissue will more accurately reflect the allelopathic agent’s presence than will juglone concentration of tissue alone. The term juglone potential accepts the multiple chemical nature of juglone found in walnut tissue and gives a precise way for quantification of allelopathic action. For example, juglone potential in black walnut leaves decreases over the growing season. Figure 4.

Finding Juglone

Black walnut is a native tree of Georgia and the Eastern United States. It contains some of the largest amounts of, and concentrations of, juglone potential of any walnut. It usually grows on moist, well drained sites where soil is deep and rich. These sites have a lot of plant competitors. The black walnut can effectively grow here because of its use of chemical warfare.

The walnut family has many members which have a significant juglone potential besides black walnut. Figure 5. By far, the highest concentrations of juglone potential are found within black walnut parts. Butternut, English walnut, pecan and the rest of the hickories, all possess much smaller levels of juglone potential. Figure 6. The fruit husks usually contain the highest concentrations of juglone potential in any species.

Escaping Home

Juglone escapes from walnuts by several ways including: leaves falling and decaying; leakage from roots; in the husks of abscised fruit; and, by erosion from rain trickling over leaves and periderm. Juglone is dissolved only a small amount in water, but only a minute amount is needed to cause problems. Juglone remains in the soil surrounding walnut roots. Other plant roots that grow near walnut roots can be stunted or killed.

Annual plants, garden vegetables, fruit trees, and some broad-leaf perennials can be severely damaged when juglone is in soil. Most grasses seem immune from juglone problems. Wherever walnut roots travel, they change the soil they move through by addition of juglone. If juglone leaks back into a walnut root, it is quickly made non-toxic again and stored.

Herbicide

There are several considerations for potential use and understanding of the ecological role for juglone. These considerations include use as herbicides, appreciating the various vectors of affect, and

changes in microflora populations. Juglone has a great herbicidal potential because of its vegetation controlling characteristics. In a walnut plantation, young walnuts usually require herbicide assistance to interfere with forbs and grasses. Pretreatment and periodic application of small amounts of juglone to walnut sites might provide control of specific species and assist in establishing walnut seedlings. In an urban setting, turfgrass lawns (like Kentucky bluegrass, for example) might be maintained relatively broadleaf weed free because many turf species tolerate juglone well.

The use of natural toxins as herbicides is ideal because of the specificity of action, presence of decomposition processes in the soil providing a short active life, and in some cases, ease of production. Instead of producing a near-total elimination of unwanted species on a site, natural ecological processes can be magnified by chemically stressing unwanted species, thereby promoting crop-species growth. Unfortunately, juglone is highly toxic in a concentrated or purified form, even in the vapor phase.

Like / Dislike

Some plants are susceptible to juglone poisoning while some plants grow well around walnuts. Sensitive plants include many domestic grapes, tomatoes, root-crop vegetables, blackberries, hackberry, birch, willow, cottonwood, pine, basswood, cypress, and many types of broad-leaved forbs. Gardens and nurseries should be kept away from black walnuts. Walnut poisoning causes foliage yellowing and wilting due to damaged roots.

Some plants which get along well with walnut include most grasses, oak, red cedar, hawthorn, black cherry, locust, maple, and some types of clover. Plants that grow in the uplands and have allelopathic chemicals of their own generally grow well with walnut. Plants that grow in wetlands are usually susceptible to walnut poisoning. Plants under other kinds of stress, like drought, are more susceptible to juglone problems.

Delivery System

The vectors for the toxic effect of black walnut on other vegetation are the fruit, leaves, and roots. Leakage from the root symplast of hydrojuglone and its oxidation to juglone produces a walnut rhizosphere that is highly antagonistic to many plant root systems. Close association with walnut roots by juglone-sensitive species probably causes respiration interference, changes in membrane permeability, and inhibition of elongation. The wilting symptom found in some juglone-sensitive plants involves the slowed movement of water across the root into vascular tissue and/or the disruption of hormonal control mechanisms.

Walnut leaf litter decomposes quickly, producing an organic layer with a yearly influx of juglone. Walnut fruit, which have large juglone potentials, may substantially alter a site chemically upon abscission and decay. Rain drip from the tree crown may further chemically antagonize other species by polluting rainfall with minute amounts of juglone. Although juglone is only slightly soluble in water, the toxicity symptoms appear at low concentrations.

Those species that grow in the presence of walnut roots still have difficulty surviving within the dripline of the tree, suggesting that rhizosphere adaptation systems of walnut-associated species depend upon the avoidance of toxin or biological negation of its effects. Species with strong chemical interference components seem to grow well together because of the maintenance of a specific rhizosphere environment around their own roots.

Living Together

Macroflora speciation and distribution in a walnut stand has been shown to be greatly affected by juglone. More study is needed concerning microflora dynamics within the root zone. The evolutionary pressure on microorganisms associated with walnut to adapt to the presence of juglone is strong. Select

mycorrhizal fungi and rhizosphere-inhabiting microbes have been shown to be highly adapted to walnut tree control zones and the presence of juglone.

In the forest, as each tree species chemically alters its site to increase potential for interference, the sphere of chemical influence around a tree will affect any species present and its population dynamics. As a tree varies in its chemical nature over its range, so will macrobial and microbial populations found associated with it. Out of ecological necessity, the greater chemical coadaptation component of interference a tree species possesses, the more specialized will be the species associated with it.

Killer Heritage

Many plants will not grow well around black walnut or on sites where black walnut has recently lived. Some trees like red maple, willow, and apple will not perform well on sites recently occupied by living black walnut roots. One full growing season after walnut removal is usually enough to eliminate most of the juglone from a healthy soil. Several seasons may be needed to eliminate residual juglone in poorly drained, compacted or flooded sites, or sites with organic soils.

For very juglone susceptible plants, like tomatoes, potatoes, and peppers, even walnut mulch, or rain dripping from foliage, can be damaging. Leaves, twigs, fruit husks, and wood chips from walnut trees should be well aged or completely composted before adding to a garden or landscape, if at all. An oxidation and aging process first converts all the hydrojuglone into toxic juglone, and further aging and oxidation under moist aerated conditions break juglone apart into non-toxic pieces. Black walnut stumps should be ground-down or removed from a site. Any chips or sawdust should be taken away for composting.

Walnut crowns should never overhang gardens or sensitive plants. Walnuts near gardens may need a plastic root barrier placed at the edge of the garden. This will keep walnut roots away from sensitive garden plants. Cutting walnut roots without installing a plastic root barrier at least 16-24 inches deep, will cause even more walnut roots to enter a garden over the next two years.

People Problems

Touching black walnut tree parts and removing nut husks will not normally injure people. Some people are very sensitive to the toxin. To these people, just small amounts of black walnut sawdust on the skin can cause small blisters. Animal bedding should not contain black walnut bark, leaves, or wood chips or dust. The husk and foliage of black walnut, or their extracted solutions, should not be ingested, applied to the skin, or used in herbal medicines. Clothes naturally dyed with black walnut husks should be washed once after natural dyeing before wearing, and avoided altogether by people with known sensitivity.

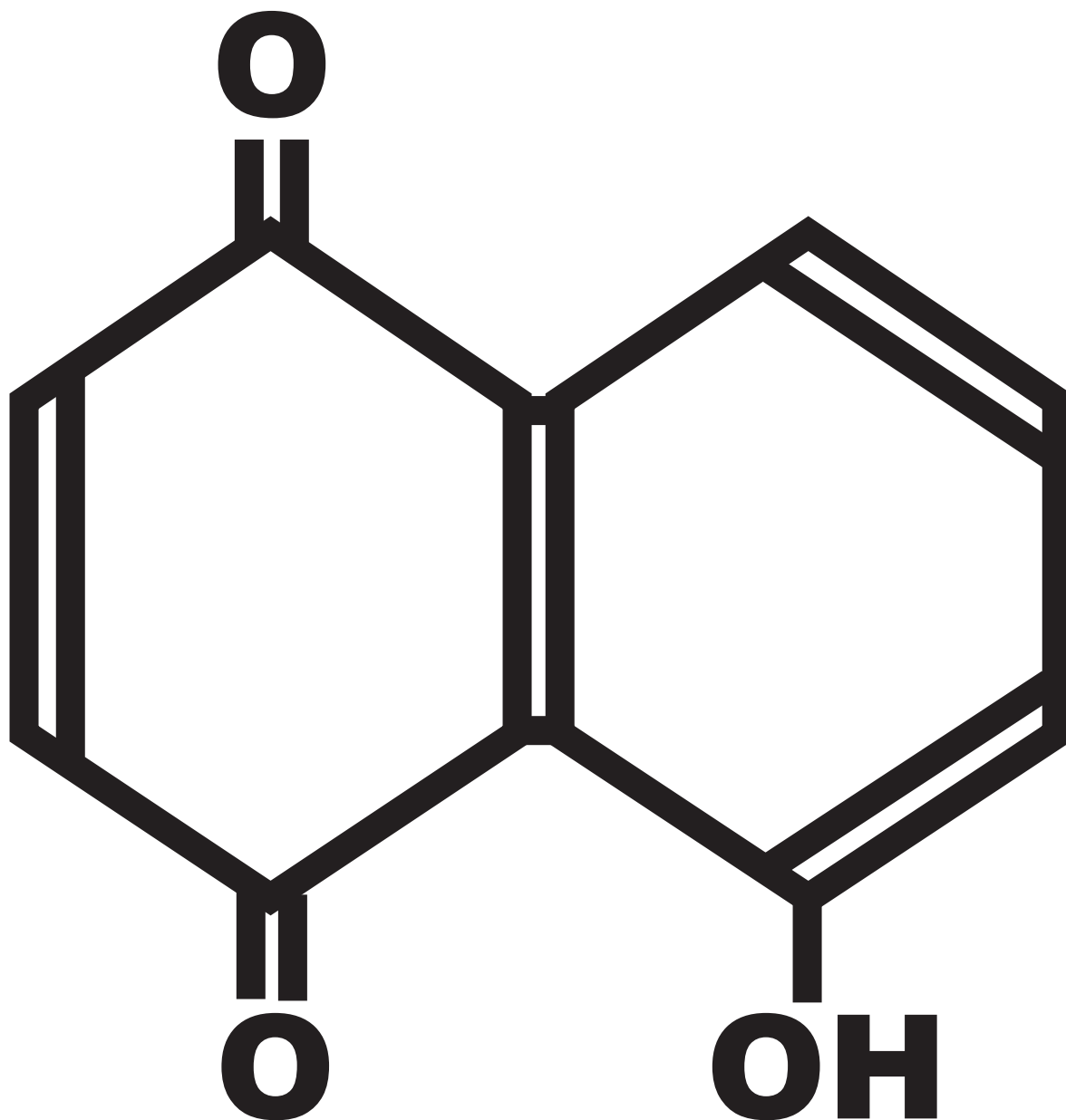
Conclusions

The ecological significance of juglone generation by black walnut requires more study. Many questions remain in approaching the synecology of black walnut allelopathy. Black walnut is a great tree which has developed a unique way to live among surrounding competitors. Instead of racing to colonize and control space filled with essential resources, the walnut bends the ecological rules to try and control its neighbors.

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5-hydroxy-1,4-naphthoquinone
or
5-hydroxy-1,4-naphthalenedione
= juglone

Figure 1: Chemical diagram of juglone -- a 10 carbon, 3 oxygen, and 2 ring compound.

tissue type	relative amount
fruit	100
whole leaf	57
individual leaflets	13
leaf stem (rachis)	44
vegetative buds	14
flower buds	23
male flowers (unopened catkins)	2
phloem (older than 1 year)	5
phloem (less than 1 year old)	2
xylem	0.3
leaf primordia	0.0

Figure 2: Relative amount of juglone potential in various tissues of *Juglans nigra*.

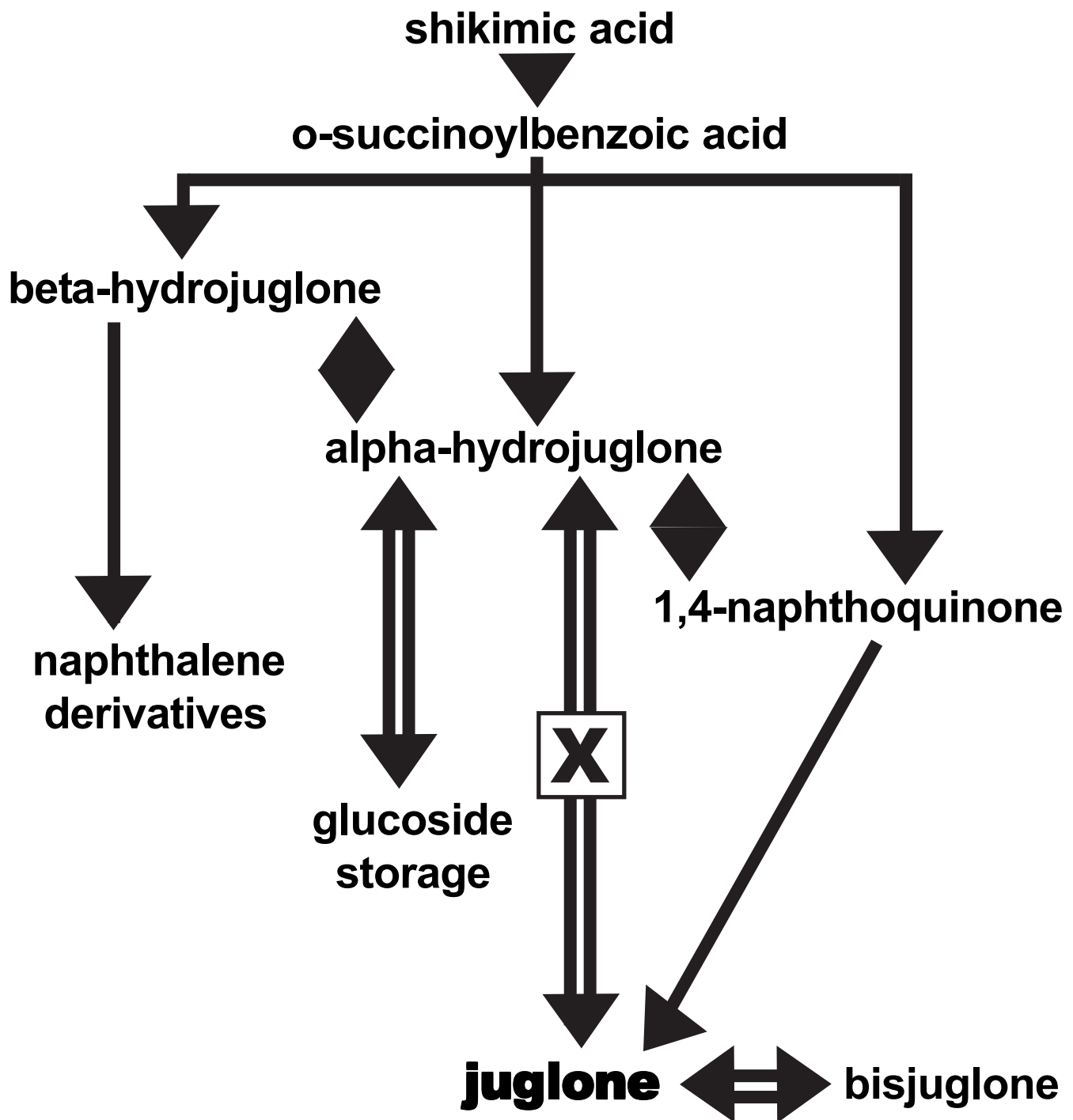


Figure 3: Metabolic synthesis net leading to juglone in *Juglans* species. Reaction **X** is the key reversable pathway.

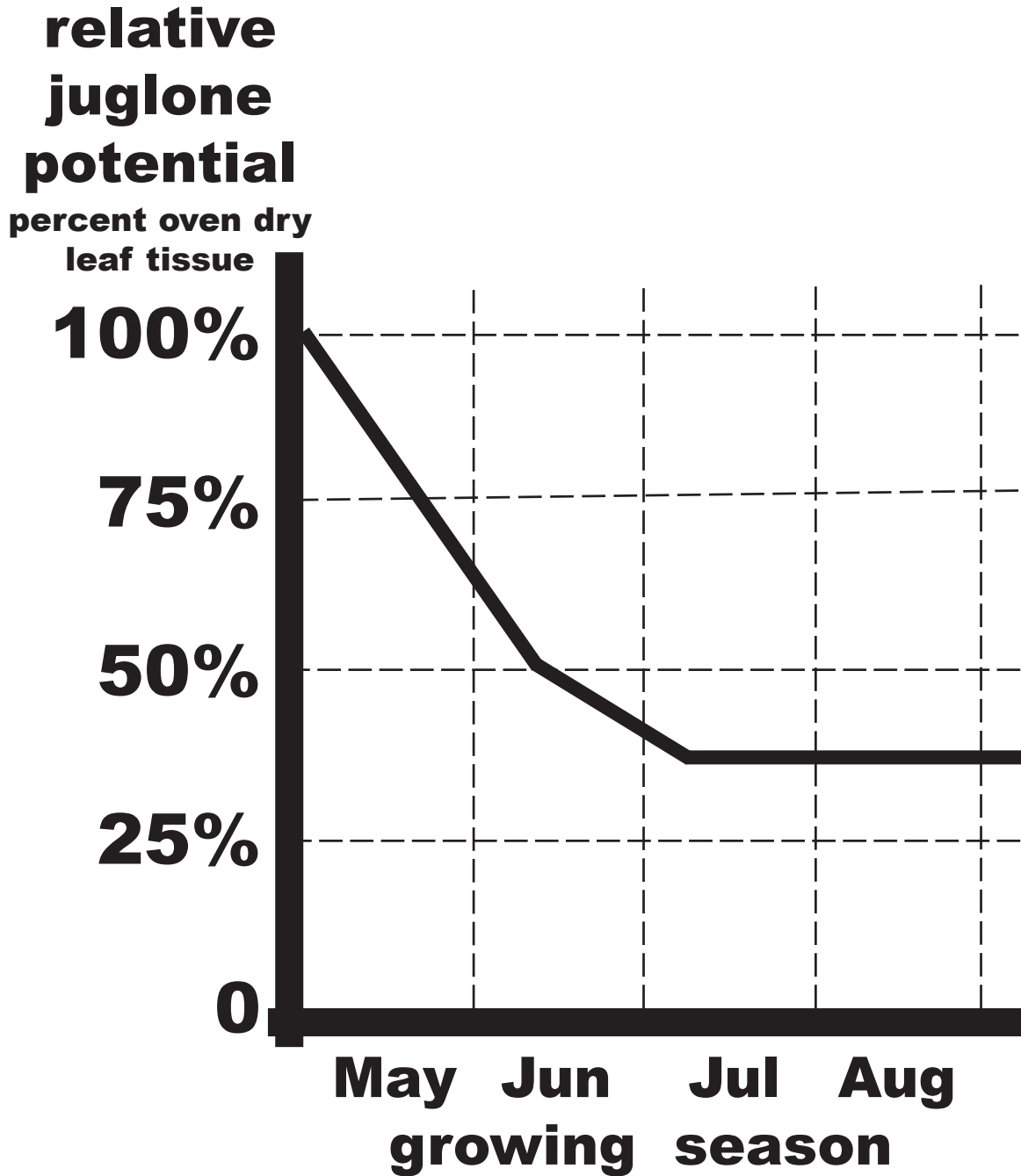


Figure 4: Relative juglone potential (percent) changes throughout crown leaf tissues for plantation grown *Juglans nigra* over a growing season.

scientific name	common name
<u>Juglans cinerea</u>	butternut
<u>Juglans nigra</u>	black walnut
<u>Juglans regia</u>	English walnut
<u>Carya cordiformis</u>	bitternut hickory
<u>Carya glabra</u>	pignut hickory
<u>Carya illinoensis</u>	pecan
<u>Carya ovata</u>	shagbark hickory
<u>Carya tomentosa</u>	mockernut hickory

Figure 5: Common members of the *Juglandaceae* which generate measurable juglone potential. All species in the genera of *Juglans* and *Carya* will generate measurable amounts of juglone potential.

scientific name common name	leaves	fruits
<u>Juglans nigra</u> black walnut	68	100
<u>Carya ovata</u> shagbark hickory	8	57
<u>Carya illinoensis</u> pecan	16	34

Figure 6: Relative juglone potential in leaves and fruits (primarily green husks) for three tree species in *Juglandaceae*. Note, black walnut green fruit husk juglone potential is set at 100%.