

AOSA and ISTA Tetrazolium Testing References:

Excerpts from methods and evaluation statements (Some paraphrased for easier comparison):

(Prepared in 2004 by Victor Vankus, US Forest Service Seed Laboratory)

Methods similar throughout both handbooks. Differences listed below.

AOSA often describes two methods. If one of the methods matches the ISTA method, the prescriptions are considered similar and not different. A note is made that a second method is described. (e.g. Cornus)

	AOSA	ISTA
Aceraceae <i>Acer</i> spcs.	remove pericarp, cut longitudinally removing part of cotyledons cut to evaluate allowable damage: less than 1/2 cotyledon	remove from pericarp, cut off a piece of seed seed coat or cut opposite radicle, soak another 3 hours and remove seed coat
Agavaceae <i>Cordyline</i>	imbibe on moist paper towels preparation and evaluation similar	soak 18 hours
Berberidaceae <i>Berberis</i> <i>Mahonia</i>	0.1% overnight 30-35 cut longitudinally, leaving cotyledons evaluation similar	1% 18 hours 30 cut transversely, stain, cut
Betulaceae <i>Alnus</i>	cut longitudinally off center or remove distal end of cotyledons, stain, cut	remove distal end of cotyledons, stain, extract embryo
Bignoniaceae <i>Catalpa</i>	methods similar	
Caesalpinaceae (Fabaceae)		
<i>Cercis</i> <i>Gleditsia</i> <i>Gymnocladus</i>	soak/imbibe between blotters (report hard seed), cut through cotyledons, stain, cut to evaluate if necessary allowable damage: radicle (slight), none on	dry cut transversely, soak in water, cut sides, stain, extract embryo allowable damage: radicle tip, 1/2 or 1/3 distal end cotyledon
Caprifoliaceae <i>Lonicera</i> <i>Sambucus</i> <i>Viburnum</i>	allowable damage: none, embryo and endosperm completely stained	allowable damage: none, except small necrosis on endosperm opposite embryo
<i>Symphoricarpos</i>	cut longitudinally through drupe after soaking and prior to staining	cut off distal 1/3 after soaking and prior to staining, cut longitudinally

Cornaceae

*Cornus* methods similar, second alternative method described use the best stained embryo for evaluation negates the need to record second viable embryo  
evaluation similar although small unstained necroses on periphery of endosperm is acceptable number of *Cornus mas* staining time 48 hours

Cupressaceae

*Calocedrus* soak, cut longitudinally, stain allowable damage: none, except for small surface necroses on female gametophyte tissue dry cut transversely at both ends, stain using low pressure, cut longitudinally and extract embryo to evaluate allowable damage: none except for small necrosis on outer endosperm  
*Chamaecyparis* soak, cut laterally, stain, cut longitudinally to expose embryo for evaluation dry cut transversely (both ends for *C. thyoides*), stain, cut  
*Cupressus* allowable damage: none, except for small surface necroses on female gametophyte longitudinally and expose embryo to evaluate  
*Juniperus*  
*Thuja*

Ebenaceae

*Diospyros* clip radicle end of dry seed, soak in 400 ppm GA<sub>3</sub> 48-72 hours, cut longitudinally through seed, stain, allowable damage: endosperm may or may not be stained soak, cut seed along distal end and two edges, stain, cut longitudinally to evaluate allowable damage: none

Eleagnaceae

*Eleagnus* methods similar, second alternative method described

Ericaceae

*Arbutus* methods similar  
*Vaccinium*

Fabaceae

*Amorpha* methods similar soak/imbibe between blotters (report hard seed), cut through cotyledons, stain, cut to evaluate if necessary allowable damage: radicle soak, remove pods, cut distal 1/3, stain, remove seed coat to evaluate

*Robinia* dry cut transversely, soak in water, stain, remove seed coat for evaluation

*Wisteria* allowable damage: radicle tip, 1/2 soak, remove seed coat, stain allowable damage: radicle tip, 1/3 distal area of cotyledons, 1/2 if

Fagaceae

<p><i>Castanea</i> <i>Fagus</i> <i>Quercus</i></p>	<p>methods similar, soak before initial cut for <i>Castanea</i> and <i>Fagus</i> cut <i>Fagus</i> longitudinally leaving seed intact at cotyledon end for staining</p>	<p>dry cut transversely, remove the pericarp, soak in water, cut longitudinally, remove seed coat if possible, stain allowable damage: radicle (1/3)</p>
<p>Ginkgoaceae <i>Ginkgo</i></p>	<p>methods similar crack, puncture inner seed coat, soak seed in water, cut longitudinally adjacent to embryo, stain allowable damage: none except for small surface necroses not in contact with</p>	<p>crack, cut longitudinally adjacent to embryo, stain allowable damage: none</p>
<p>Hamamelidaceae <i>Hamamelis</i></p>	<p>methods similar allowable damage: radicle tip unstained for less than 1/3 length, 1/3 or less of distal end or sides of cotyledons</p>	<p>allowable damage: none</p>
<p><i>Liquidambar</i></p>	<p>soak, cut longitudinally leaving seed intact at cotyledon end, stain allowable damage: radicle tip unstained for less than 1/3 length, 1/3 or less of distal end or sides of cotyledons</p>	<p>soak, cut seed coat at distal end and sides, stain, expose embryo to evaluate allowable damage: none</p>
<p>Hippocastanaceae <i>Aesculus</i></p>	<p>soak, cut laterally, stain, cut longitudinally to evaluate</p>	<p>dry cut longitudinally, stain half with the embryo axis</p>
<p>Juglandaceae <i>Juglans</i></p>	<p>methods similar crack dry nuts, soak, stain, remove seed coat to evaluate second alternative method described</p>	<p>crack dry nuts, soak, remove seed coat, stain, evaluate</p>
<p>Magnoliaceae <i>Liriodendron</i></p>	<p>methods similar note on second seed per unit and the need to make a second cut during preparation</p>	
<p><i>Magnolia</i></p>	<p>soak, cut laterally at distal end, stain, cut longitudinally to stain</p>	<p>soak (remove pulpy seed coat if present), cut woody seed coat at distal end and sides, stain, cut</p>
<p>Malvaceae <i>Hibiscus</i></p>	<p>methods similar preparation cuts slightly</p>	
<p>Oleaceae <i>Fraxinus</i> <i>Syringa</i></p>	<p>soak, cut longitudinally adjacent to embryo, stain, cut longitudinally to evaluate</p>	<p>remove pericarp of dry seed (<i>Fraxinus</i>), soak, cut longitudinally on both sides of the seed to open</p>
<p>Pinaceae</p>		

<p><i>Abies</i>  <i>Cedrus</i>  <i>Larix</i>  <i>Picea</i>  <i>Pinus</i>  <i>Pseudotsuga</i>  <i>Tsuga</i></p>	<p>methods similar  alternative second preparation cut described</p>	<p>ISTA prescribes a dry cut for staining preparation cutting transversely at both ends (<i>Abies</i>)</p>
<p>Platanaceae  <i>Platanus</i></p>	<p>methods similar  allowable damage: none, radicle tip unstained acceptable and less than 1/3 distal end of cotyledon or total area</p>	<p>allowable damage: none except for small necroses on endosperm not connected to embryo cavity</p>
<p>Proteaceae  <i>Grevillea</i></p>	<p>methods similar    cut longitudinally to</p>	<p>extract embryo to evaluate</p>
<p>Ranunculaceae  <i>Clematis</i></p>	<p>methods similar    imbibe on moist filter paper or between blotters prior to</p>	<p>soak prior to preparation</p>
<p>Rhamnaceae  <i>Rhamnus</i></p>	<p>methods similar    imbibe on moist filter paper or between blotters prior to</p>	<p>soak prior to preparation</p>
<p>Rosaceae  <i>Amelanchier</i>  <i>Crataegus</i>  <i>Malus</i>  <i>Physocarpus</i>  <i>Pyrus</i>  <i>Rosa</i>  <i>Rubus</i>  <i>Sorbus</i>  <i>Prunus</i></p>	<p>methods similar    soak, cut laterally at distal end, second soak, extract embryo prior to staining</p> <p>methods similar</p>	<p>cut dry seeds (<i>Crataegus</i>, <i>Rosa</i>) and extract embryo after staining to evaluate (<i>Amelanchier</i>, <i>Crataegus</i>, <i>Rosa</i>, <i>Rubus</i>, <i>Sorbus</i>)</p> <p>spread cotyledons of large seeded species prior to staining</p>
<p>Scrophulariaceae  <i>Paulownia</i></p>	<p>genus not listed    procedure for family: soak, cut laterally at distal end of cotyledons, stain, separate halves to</p>	<p>soak, remove wings, stain, extract embryo to evaluate</p>
<p>Simaroubaceae  <i>Ailanthus</i></p>	<p>soak, cut longitudinally leaving embryo intact prior to staining, stain and evaluate</p>	<p>soak, cut transversely at distal end, stain, remove pericarp and seed</p>
<p>Simmondsiaceae</p>		

<i>Simmondsia</i>	<p>methods similar</p> <p>allowable damage: radicle none, less than 1/2 of cotyledons, not near point of attachment of cotyledons optional second cut for preparation described</p>	<p>ISTA mentions a cut at the basal end during preparation for staining diagram show distal end cut which matches AOSA</p> <p>allowable damage: none</p>
<p>Taxaceae</p> <p><i>Taxus</i></p>	<p>soak, cut longitudinally, stain 6-18 hours, second longitudinal cut if necessary to evaluate</p>	<p>soak, cut transversely through endosperm to open embryo cavity, stain 24 hours, cut longitudinally to</p>
<p>Taxodiaceae</p> <p><i>Cryptomeria</i></p> <p><i>Metasequoia</i></p> <p><i>Sequoia</i></p> <p><i>Taxodium</i></p>	<p>methods similar</p>	<p>ISTA prescribes a dry cut for staining preparation</p>
<p>Tiliaceae</p> <p><i>Tilia</i></p>	<p>methods similar remove pericarp, soak, bisect or cut longitudinally to open expose embryo/embryo cavity, second cut if needed to evaluate</p>	<p>remove pericarp, soak, remove seed coat, stain, open endosperm to evaluate</p>
<p>Ulmaceae</p> <p><i>Celtis</i></p> <p><i>Ulmus</i></p>	<p>soak, cut through endocarp to expose seed and cotyledons, stain, cut</p> <p>methods similar cut seed to evaluate</p>	<p>crack stones, soak, remove seed coat, stain, extract embryo to evaluate</p>
<p>Vitaceae</p> <p><i>Parthenocissus</i></p>	<p>soak, cut laterally, stain 18-24 hours, expose embryo by gradually slicing through the endosperm</p>	<p>soak, cut longitudinally through the center, nearly the embryo cavity, stain 48 hours, expose embryo by gradually slicing through the</p>
<i>Vitis</i>	<p>methods similar</p>	