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Differential Sensitivity of *Juglans hindsii, J. regia,* Paradox Hybrid, and *Pterocarya stenoptera* to Waterlogging[1]

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Abstract. **Seedlings of *Juglans hindsii* Jeps. and *J. regia* L. reacted similarly and were much more sensitive to waterlogging at root temperatures of 33°C than those of *Pterocarya stenoptera* DC. At 23°C, *J. regia* expressed symptoms of waterlogging earlier than *J. hindsii.* Paradox plants, hybrids between the 2 walnut species, were more tolerant than *J. hindsii* but are still considered highly sensitive to anaerobiosis. These results support the contention that use of *J. regia* seedlings as rootstocks to avoid blackline introduces greater potential for damage if soils become saturated. Some plants of each type which demonstrated increased tolerance have been selected. Levels of abscisic acid, or changes therein did not appear to be useful parameters in screening walnut seedlings for tolerance to waterlogging. Phenolic compounds decreased in roots of waterlogged plants. Although the magnitude of change in phenols was the same in *Juglans* and *Pterocarya* plants, it occurred over a much longer period with the latter. Phenols lost from roots may be a secondary phenomenon but contribute to hypersensitivity of *Juglans* to waterlogging.**

Seedlings of Northern California Black Walnut, *Juglans hindsii*, were almost exclusively the walnut rootstock used in California until about 1955. Since then, some others have been planted including: the Persian or English walnut, *J. regia,* the Paradox walnut (*J. hindsii* × *J. regia*), and to a very limited extent, the Chinese wingnut, *Pterocarya stenoptera.* Each of these rootstocks has distinct advantages and disadvantages (13). A notable disadvantage of both *J. hindsii* and Paradox rootstocks is the occurrence of blackline when topworked with *J. regia* scions. Blackline is a graft union disorder which has devastated certain major districts (12) and which appears to be spreading (8).

Because blackline has never been observed on trees with *J. regia* rootstocks, renewed interest in this species exists (8), and cultivars propagated on *J. regia* seedlings are being planted commercially. Seedlings of *P. stenoptera* do not offer an effective means of avoiding blackline because of incompatibility with most *J. regia* cultivars and susceptibility to blackline (8, 13). Even though blackline apparently can be avoided through use of *J. regia* rootstocks, it is entirely possible some other feature could exist which might be equally undesirable. One characteristic of major importance with walnut rootstocks is their sensitivity to excessive soil moisture (waterlogging, flooding, fluctuating water tables). On the basis of subjective information, *J. hindsii* is considered to be intolerant to such conditions, Paradox and *P. stenoptera* tolerant, but the response of *J. regia* is unknown (13). Because of the relationship between sensitivity of *J. hindsii* and Paradox, it is frequently said that

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J. regia must be more tolerant.

The importance of determining the reaction of *J. regia* seedlings to waterlogging was emphasized in 1974. Extreme damage or death of many thousands of trees, mostly on *J. hindsii,* was attributed to late spring flooding and/or seepage from rivers in several California walnut districts. It thus seemed appropriate to develop objective information on the major walnut rootstocks, especially *J. regia,* to potentially unfavorable root environments. Responses of walnut rootstocks were not included in a recent review of waterlogging of fruit trees (11), but *J. regia* has been reported to be more sensitive than *J. nigra* L.(14). The objective of this study was to compare the sensitivity to waterlogging of *J. regia* seedlings with those of *J. hindsii,* Paradox, and *P. stenoptera.*

Materials and Methods

Earlier work with *Prunus* species employed a testing procedure which allowed rapid, objective determination of sensitivity to waterlogging (10). Paradox plants (*J. hindsii* × *J. regia*) and seedlings of *J. regia, J. hindsii,* and *P. stenoptera* were grown in sterilized sand in 1 quart milk cartons in the greenhouse. When 6 to 12 weeks old, the cartons containing the plants were placed in polyvinyl chloride containers slightly wider and deeper than the cartons. The containers were then placed in a large controlled temperature bath in the greenhouse. Tap water was added to the containers and maintained at a level about 2.5 cm above the level of the sand. The containers prevented mixing of water surrounding the roots with that of the temperature bath. Bath temp was maintained at 23° or 33°C. Air temp were regulated only within broad limits throughout the year from about 10 to 16° at night to 22 to 32° during the day. In practically all cases any 2 phenotypes were compared directly, being arranged in alternate rows in the same bath at the same time, usually 20 seedlings of each.

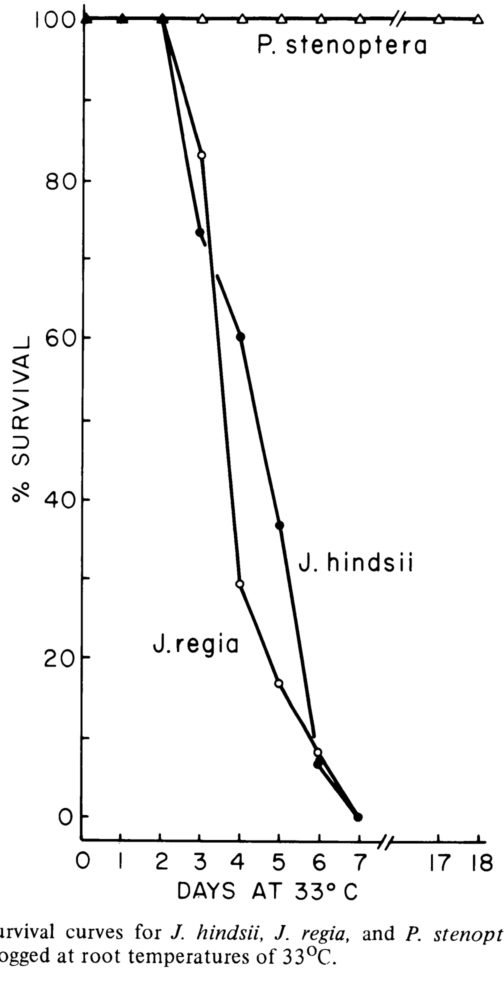

Fig. 1. Survival curves for *J. hindsii, J. regia,* and *P. stenoptera* plants waterlogged at root temperatures of 33°C.

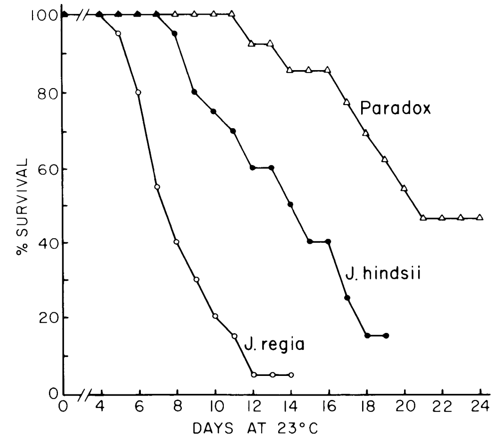

Fig. 2. Survival curves for *J. hindsii, J. regia* and Paradox plants waterlogged at root temperatures of 23°C.

The time of appearance of symptoms of waterlogging was recorded, and the plants were removed, drained, and held for observation in the greenhouse. Wilting of leaves was the predominant symptom, but in some cases leaf chlorosis and/or the appearance of necrotic patches on the laminae preceded wilting and was used as the indicator of damage.

Analysis of variance of length of survival of waterlogged plants was performed, and the Scheffé method for multiple comparisons used to test differences between means (7).

For abscisic acid (ABA) determinations, 3 leaves each were taken from non-treated and from flooded plants at various times throughout each experiment. Sample leaves were immediately weighed, cut into pieces, extracted with methanol, and the methanol extracts fractionated as previously described (9). ABA was estimated using a modified wheat coleoptile bioassay (3).

With some experiments the amounts of phenolic compounds in root systems were determined. When symptoms appeared in the shoots, roots were washed free of sand, separated into taproot and lateral plus fine roots, weighed, cut finely and immersed in boiling methanol for 10 min. After homogenization the samples were extracted with methanol (2) and total phenols determined using a modified Folin-Denis method (5). Roots of control plants were sampled at the same time and similarly processed.

Results and Discussion

The effect of waterlogging at 33°C on survival of seedlings of *J. hindsii, J. regia* and *P. stenoptera* is shown in Fig. 1. With the 2 walnut species the 1st plants were affected on the 3rd day, and all had succumbed by the 7th day. None of the *P. stenoptera* plants had wilted after 18 days when half of them were removed from treatment. The remainder were left in the bath 26 days without wilting. This does not imply that there was no effect of waterlogging on the wingnut plants. There was very gradual development of leaf chlorosis and some abscission, both progressing from the base of the plants. Many of the fine roots were killed. After removal of the wingnut seedlings from the bath after either 18 or 26 days they slowly recovered. This was not the case with walnut seedlings treated at 33°C.

Sensitivity to waterlogging is temperature dependent (1). Differences in sensitivity among *Prunus* species found at lower temperatures were not as evident at higher ones (10). Thus, seedlings of the 2 walnut species and Paradox plants were waterlogged at a lower temperature, 23°C, to determine if a difference between them could be resolved. Survival curves typical for the 3 sorts of seedlings are shown in Fig. 2. More time was required for initiation of symptoms at the lower

Table 1. Results of analysis of variance and tests for differences between means for certain levels of survival of *J. hindsii*, *J. regia*, and Paradox plants waterlogged at 23°C.

Species		Survival 80%	50%	20%
J. regia	$\bar{x}^z$	5.32	6.61	8.19
	σ	0.36	0.28	0.26
	n	88	220	352
J. hindsii	$\bar{x}$	10.77	13.30	15.36
	σ	0.31	0.24	0.23
	n	119	301	459
Paradox	$\bar{x}$	14.91	19.44	22.90
	σ	0.70	0.53	0.69
	n	23	62	50
		Differences between means (days)		
J. hindsii vs. *J. regia*		5.45 ± 1.17^y	6.69 ± 0.91	7.17 ± 0.84
Paradox vs. *J. hindsii*		4.15 ± 1.95	6.13 ± 1.42	7.53 ± 1.77
Paradox vs. *J. regia*		9.60 ± 1.95	12.82 ± 1.47	14.71 ± 1.79

^{z}Days.

^{y}95% confidence limits.

temperature. Similarly, at 23° the time required for a high proportion of seedlings to become affected was increased. The time for any given percentage of *J. regia* seedlings to succumb was shorter than for those of *J. hindsii* which in turn were shorter than Paradox plants. Considerable seedling variability was also evident with overlap among the most sensitive individuals of one sort with the most tolerant of another. Curves in Fig. 2 do not reach 0% survival since one objective was to select individual plants which appeared to possess superior tolerance to waterlogging. Cultures from roots of waterlogged plants, occasionally made to detect the presence of pathogenic fungi, were negative.[2]

The same survival patterns and relationships were obtained from treatments using seedlings from, respectively 5 cultivars of *J. regia* (Serr, Eureka, Payne, Ashley, Franquette), 7 unnamed clones of *J. hindsii,* and 2 sources of Paradox. Some seedling lots were treated more than once so that in all 440 *J. regia,* 574 *J. hindsii* and 124 Paradox plants were included. Analysis of variance of the time required for certain percentages of seedlings to wilt and results of tests for differences between means are given in Table 1. Differences between all pairs of means were significant at the 5% level. We conclude that on the average, seedlings of *J. regia* are more sensitive to waterlogging than those of *J. hindsii.* Paradox hybrids are less sensitive than either species parent. Paradox thus display overdominance with respect to inheritance of tolerance to anaerobiosis.

Conclusions based upon field observations of comparative tolerance of *J. hindsii* and Paradox (13) are confirmed. The supposition that *J. regia* must be more tolerant based on such observations of Paradox and *J. hindsii* is shown here to be incorrect. A few individuals of each type are equally sensitive. Nevertheless, some advantage could be gained by employing Paradox rootstocks where increased potential for saturated root environments prevails.

Table 2. Abscisic acid concn in leaves of control and waterlogged plants of *J. hindsii, J. regia,* and *P. stenoptera.*^{z}

Species	ABA (μg/g fresh wt) Waterlogged 33°	23°	Control
J. hindsii	1.18		.35
		1.38	.33
J. regia	.59		.46
		.23	.29
P. stenoptera	.98		.98

^{z}Except with *P. stenoptera,* leaves of waterlogged plants expressed incipient symptoms.

Table 3. Relative content of phenolic compounds in roots of control and waterlogged plants of *J. hindsii, J. regia,* and *P. stenoptera* (33°C).

Species	Optical density units Waterlogged	Control
J. hindsii		
Fine roots	.052	.22
Taproots	.11	.22
J. regia		
Fine roots	.052	.17
Taproots	.24	.30
P. stenoptera		
Fine roots	.012	.13
Taproots	.047	.14

Even though significant differences exist among the 3 sorts of *Juglans* seedlings, these differences are relatively small. All would be regarded as highly sensitive (11). Much greater tolerance exists with *P. stenoptera.* Wingnut has little potential as a walnut rootstock but would seem useful in comparative studies of mechanisms controlling tolerance.

The greater sensitivity exhibited by *J. regia* seedlings provides a note of caution for their use as rootstocks to avoid blackline where waterlogging potential exists. Where a few days difference in duration of anaerobic conditions could occur with seepage or flooding in spring, more damage would be expected with *J. regia* rootstocks than with either *J. hindsii* or Paradox. This would be particularly true with heavy soils or with faulty irrigation during summer when elevated soil temperatures prevail. However, the choice here may be between risk of waterlogging damage versus almost certain decline and death if trees became affected with blackline.

In most treatments, plants appearing unaffected when 80% or more of the others of the same seed lot had succumbed were drained and held as potential survivors. These plants behaved in different ways – a few rapidly wilted and died; leaves on others gradually senesced and abscised. With the latter there was considerable variation in extent, ranging from complete defoliation to practically no change. Such plants had obviously been affected by waterlogging but more slowly and to a lesser extent than most others.

At 6 weeks after removal of the potential survivors from waterlogging the plants were washed free of sand and their roots examined. If any new root formation had occurred, they were repotted in soil-fir bark mix and maintained for further recovery and, ultimately, planting in the field. About 60% of those selected as potential survivors have been repotted to date. The remainder did not survive the 6-week holding period. Repotted survivors were planted in the nursery after undergoing either winter chilling or 8-10 weeks at about 3-7°C in a cold room. Survival in the nursery has been high with growth to heights of 1-2 m being common in one growing season.

Selected individual plants with apparently superior tolerance to saturated root environments offer potential for improving walnut rootstocks. Such plants represent a gene pool having a small genetic advantage with regard to tolerance to waterlogging. Also, if procedures for vegetative propagation were available, clonal rootstocks could be produced for sites having increased frequency of becoming saturated. Two of the major walnut producing areas in California are characterized by either heavy soils or close proximity to rivers. The ability to survive even a few more days than the average could have dramatic effects. This was illustrated by the occasional tree which survived saturated soils in spring 1974 where all others in the immediate proximity were killed or very severely damaged. Had these orchards been planted with clonal rootstocks with superior tolerance much less damage might have occurred.

Water stress is apparently one of the phenomena involved in symptom expression, and water stress has been shown to cause dramatic increases in ABA in leaves (16). To determine whether ABA was involved in symptom development and if it could be employed in screening of seedlings, the levels of this regulator were determined in leaves from control and treated plants. Sampled leaves from treated plants were pale green and slightly flaccid but not wilted. With *J. hindsii,* leaves of waterlogged plants at both 33°C and 23°C contained several times more ABA than those from the controls (Table 2). With *J. regia,* there was little difference in ABA due to waterlogging. ABA was high in *P. stenoptera* leaves, and there was no difference between treated plants and the controls.

The increase in ABA in *J. hindsii* leaves at 33°C, which presumably would have effected stomatal closure with resultant reduced water loss, was not sufficient to delay symptom development. The fact that ABA increased in *J. hindsii* leaves but did not do so in *J. regia* may in part explain the greater tolerance of many of the *J. hindsii* plants at 23°C. Although ABA did not increase in *P. stenoptera* leaves, naturally high levels in nonstressed plants may be related to the high tolerance of these plants. Although ABA may be involved in symptom expression, the objective here was to ascertain whether levels of ABA or changes therein might indicate effects of waterlogging before gross morphological symptoms developed. Even though ABA increased in *J. hindsii* leaves with incipient symptoms, the lack of similar behavior with *J. regia* precludes general use of this parameter as a screening test. It may be that *J. regia* plants are similar to the wilty tomato mutant which contains low levels of ABA and wilts readily as the stomata remain open (15).

Root cells have been shown to release their contents during anaerobiosis (6). Loss of selective permeability of membranes was thought to lead to hydrolysis of cyanogenic glycosides with resultant HCN contributing to sensitivity of *Prunus* to waterlogging (10). Although walnut does not contain cyanogenic glycosides (unpublished), release of vacuolar phenolic compounds would be expected to be inhibitory to cellular metabolism through complexing with and denaturation of proteins.

Decreases in phenolic substances were found in both the fine roots and tap roots of waterlogged plants (Table 3). The same pattern was found with *P. stenoptera* as with the 2 walnut species. The low levels in roots of the 2 walnut species were reached in 3-7 days as compared to 22-26 days with wingnut. The lower inherent levels of phenols in *P. stenoptera* roots and a loss, similar in magnitude to that in *Juglans,* determined after much longer treatment, apparently minimized their influence in symptom development.

Although the inhibitory effect of released phenols is only suggested here, some observations lend support to this view. In many cases with treated plants a black coloration has been observed in xylem of stem and petioles. Also, localized necrotic areas often appeared on laminae before wilting. The same observations have been made with a few trees in the field which were damaged by waterlogging. It is postulated that some of the phenolic substances lost by cells in the root enter the transpiration stream and are transported to above-ground parts of the plant where they can cause damage. Such damage would be in addition to that which likely occurs in and adjacent to cells from which the release took place.

It is not suggested that the release of phenolics is a primary phenomenon involved in sensitivity to waterlogging. Initial effects would seem to be those already proposed (10) in that anaerobiosis inhibits production and transfer of energy required for maintenance of membranes. Support for the involvement of the respiratory mechanism as a primary influence in sensitivity is gained by the proposal that metabolic pathways shift to production of ethanol during anaerobiosis (4). Release of phenolics and their secondary inhibitory action could well contribute to hypersensitivity and is consistent with a similar role suggested for cynaogenic glycosides in *Prunus* (10).

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