Phloem and xylem nitrogen variability in *Quercus rubra* attacked by *Enaphalodes rufulus*

Laurel J. Haavik, Matthew P. Ayres, Erik E. Stange, Fred M. Stephen

Abstract—We analyzed nitrogen (N) content in phloem and xylem of 75 northern red oak, *Quercus rubra* L. (Fagaceae), removed in 2002–2007 from three sites within the Ozark National Forest, Arkansas, to investigate the relationship between vascular tissue N status and wood borer infestation. Populations of native red oak borer, *Enaphalodes rufulus* (Haldeman) (Coleoptera: Cerambycidae), experienced an outbreak and population crash during the sampling period. Vascular tissue N was not correlated with borer infestation density (exit holes/m^{-2}/bark surface), which suggests variation in N status of vascular tissue was not influenced by or did not influence borer success. Vascular tissue N was greater in trees sampled during winter months than in trees sampled in spring and summer months.

Résumé—Nous avons analysé le contenu en azote (N) du phloème et du xylème de 75 chênes rouges boréaux, *Quercus rubra* L. (Fagaceae), prélevés en 2002–2007 dans trois sites de la forêt nationale d'Ozark, Arkansas, afin d'examiner la relation entre le N du tissu vasculaire et l'infestation par les insectes mineurs du bois. Durant la période d'échantillonnage, les populations du grand longicorne du chêne rouge, *Enaphalodes rufulus* (Haldeman) (Coleoptera : Cerambycidae), un insecte indigène, ont connu une épidémie et un effondrement. Il n'y a pas de corrélation entre le N du tissu vasculaire et la densité de l'infestation des mineuses (nombre de trous de sortie m^{-2} de surface d'écorce), ce qui laisse croire que le statut en N du tissu vasculaire n'est pas influencé par le succès des mineuses, ni ne l'influence. Les concentrations de N du tissu vasculaire sont plus élevées dans les arbres prélevés durant les mois d'hiver que durant les mois de printemps et d'été.

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The low nutritional quality of wood relative to other plant tissues results in long development times ($\geq$1 year) for many wood-feeding Buprestidae and Cerambycidae (Coleoptera) (Nielsen 1981). Red oak borer, *Enaphalodes rufulus* (Haldeman) (Coleoptera: Cerambycidae), requires 2 years of larval feeding to complete development, and adults emerge in odd-numbered years only (Hay 1969). Arkansas oak–hickory forests (*Quercus* L. (Fagaceae), *Carya* Nutt. (Juglandaceae)) recently experienced an unexpected outbreak of *E. rufulus* (Stephen *et al.* 2001) followed by an abrupt population crash (Riggins *et al.* 2009). The outbreak was likely associated with stand maturity and several drought events (Haavik and Stephen 2010). Although drought often precedes wood-boring insect outbreaks, it is unclear whether increased insect population growth is a result of weakened host resistance, increased nutritional quality of vascular tissues, or both (White 1984). Some host trees were apparently more resistant to *E. rufulus*, in terms of tolerance and maintenance of growth rates, during

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the outbreak than others (Haavik and Stephen 2011). The role of host nutrition in the outbreak remains unknown.

The objective of this study was to examine variation in nitrogen (N) content of phloem and xylem tissue within the borer's primary host northern red oak, *Quercus rubra* L. (Fagaceae), in relation to borer colonization and infestation densities. We predicted that hosts with greater *E. rufulus* infestations would also have higher concentrations of N in vascular tissue. An increase in N could be a consequence of stress, either induced by recent drought or by borer feeding. We also expected that *E. rufulus*' initial colonization densities would be positively correlated with phloem N, *i.e.*, that fewer early instars would survive to initiate galleries within trees with low phloem N. Additionally, we examined variables related to sites, trees, and timing (*i.e.*, season and year in which trees were sampled) to explore their influence on vascular tissue N content.

Host *Q. rubra* were removed from three previously established research areas within the Ozark National Forest in Arkansas (Fly Gap, 35°45′N, 93°47′W; White Rock, 35°35′N, 93°58′W; and Oark, 35°41′N, 93°34′W (Fierke *et al.* 2005)). Seventy-five trees were harvested from 2002 through 2007. Minimum and maximum sample sizes for each site, cohort, and season combination were 4 and 7, respectively. Seasons were designated based on phenology of oak trees and life history of borers. Borers and trees were inactive in winter (December through February), trees began photosynthesizing for early wood production and bud burst in spring (March and April), borers and trees were active in late spring and summer (May through August), and only borers were active in fall (September through November) (Hay 1969; Pallardy 2008).

Trees were destructively sampled for number of exit holes on the outer bark surface (a measure of infestation) and then the outer bark was carefully scraped away to reveal initiated early-instar larval phloem galleries from the current generation (a measure of colonization) (Fierke *et al.* 2005). Populations of *E. rufulus* were at outbreak levels during development of the 2003 cohort of emerging adults and low during the 2005 and 2007 cohorts (Riggins *et al.* 2009). Prior to tree felling, diameter at breast height (DBH, 1.4 m from the ground) was recorded. Trees were then felled and a 5 cm thick basal cross-section was cut and removed from each tree bole at approximately 0.5 m above the ground and transported to the laboratory for N sampling and tree age determination.

Table 1. Pearson's correlation matrix for four variables in *Quercus rubra* trees in Arkansas: Total phloem N (%), total xylem N (%), exit-hole density (exit holes/m^{-2}/bark surface), and initiated gallery density (initiated galleries/m^{-2}/bark surface).

	Phloem N	Xylem N	Exit holes
Phloem N	—	—	—
Xylem N	0.459*	—	—
Exit holes	0.013	0.003	—
Initiated galleries	0.033	0.046	0.764*

*Indicates statistically significant relationships ($P < 0.05$).

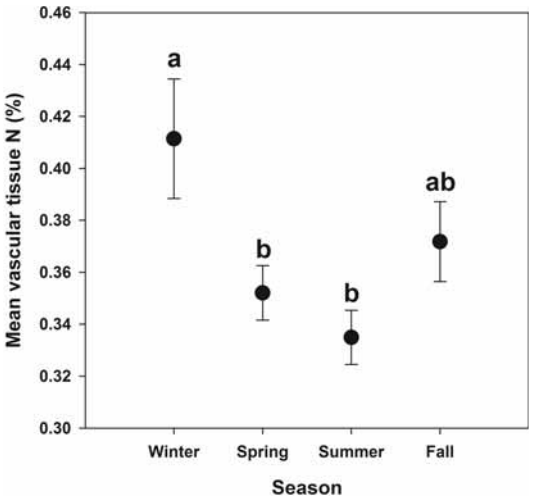
Two 1 g phloem and two 1 g xylem (sapwood) samples were extracted from opposing sides of each cross-section with a mallet and wood chisel. Samples were ground to sawdust using a Wiley Mill (<60 μm) and analyzed for total percentage N on a Carlo Erba elemental analyzer (1110 CHN Combustion Analyzer, Carlo Erba Strumentazione, Milan, Italy). Two replicate samples (one of phloem, the other of xylem) were averaged together to represent N content for each tissue at the individual tree level.

All statistical analyses were conducted in JMP 9.0 (SAS Institute Inc. 2010). Pearson's pairwise correlations were utilized to examine relationships among phloem N, xylem N, infestation, and colonization. Phloem and xylem N varied positively together (see Table 1) and thus were added together for total vascular tissue N in subsequent analysis. Stepwise multiple linear regression was used to determine whether potential predictors (tree age, DBH, site, cohort, or season) were significantly related to variation in vascular tissue N. All predictors with nonsignificant partial *F* tests were removed from the final model. Residual plots were checked for violations of model assumptions. Statistical significance

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Fig. 1. Season in which *Quercus rubra* trees were sampled in Arkansas was a significant predictor for variation in vascular tissue nitrogen (N). Letters above the bars indicate statistically significant differences according to Tukey's HSD. Sample sizes were winter (n = 13), spring (n = 24), summer (n = 20), and fall (n = 16).



was set at $P \leq 0.05$ and errors are presented as one standard error from the mean.

Phloem and xylem N ranged from 0.15% to 0.41% (mean 0.22% $\pm$ 0.01%) and 0.07% to 0.24% (mean 0.14% $\pm$ 0.01%), respectively. Exit hole and initiated gallery densities per m^2 bark surface ranged from 0.08 to 83.06 (mean 12.99 $\pm$ 2.34) and from 0.43 to 257.53 (mean 52.20 $\pm$ 7.47), respectively. Phloem N and exit-hole density were significantly positively correlated with xylem N and initiated gallery density, respectively, although no other relationships among these four variables were significant (Table 1). Vascular tissue N content varied significantly by season ($F_{3,69}$ = 4.71, P = 0.005; Fig. 1); trees sampled during winter had greater vascular tissue N than did trees sampled during spring or summer, and trees sampled during fall were not different from trees sampled during other seasons (Fig. 1).

Vascular tissue N of *Q. rubra* trees did not vary with colonization (initiated phloem gallery density) or infestation levels (exit-hole density) of *E. rufulus*, suggesting that there was no relationship between vascular tissue N and colonization or infestation. Though not revealed through this study, nutrition still may have been important in rendering *Q. rubra* trees susceptible and (or) suitable as hosts for *E. rufulus*. Another wood feeding beetle, *Anobium punctatum* (De Geer) (Anobiidae), utilized soluble N to a greater extent than insoluble N during larval growth and development (Baker *et al.* 1970), and soluble N may become increasingly available within woody tissues during soil moisture deficit (White 1984). A more complete investigation should include analysis of soluble N available in host tissue and its use by *E. rufulus* during larval development.

Timing variables were apparently important for vascular tissue N content, which varied seasonally (Fig. 1), which is consistent with increased concentration of N storage compounds during dormant months (Pallardy 2008). Xylem and phloem N contents also increased together, likely due to the close proximity of these tissues, solute exchange between them, and the high degree of N mobility among plant organs (Pallardy 2008).

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Baker, J.M., Laidlaw, R.A., and Smith, G.A. 1970. Wood breakdown and nitrogen utilisation by *Anobium punctatum* Deg. feeding on Scots pine sapwood. Holzforschung, **24**: 45–54. doi:10.1515/hfsg.1970.24.2.45.

Fierke, M.K., Kinney, D.L., Salisbury, V.B., Crook, D.J., and Stephen, F.M. 2005. Development and comparison of intensive and extensive sampling methods and preliminary within-tree population estimates of red oak borer (Coleoptera: Cerambycidae) in the Ozark Mountains of Arkansas. Environmental Entomology, **34**: 184–192. doi:10.1603/0046-225X-34.1.184.

Haavik, L.J., and Stephen, F.M. 2010. Historical dynamics of a native cerambycid, *Enaphalodes rufulus*, in relation to climate in the Ozark and Ouachita Mountains of Arkansas. Ecological

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Entomology, **35**: 673–683. doi:10.1111/j.1365-2311.2010.01225.x.

Haavik, L.J., and Stephen, F.M. 2011. Factors that affect compartmentalization and wound closure of *Quercus rubra* infested with *Enaphalodes rufulus* (Coleoptera: Cerambycidae). Agricultural and Forest Entomology. In press. doi:10.1111/j.1461-9563.2011.00522.x.

Hay, C.J. 1969. The life history of a red oak borer and its behavior in red, black, and scarlet oak. Proceedings North Central Branch: Entomological Society of America, **24**: 125–127.

Nielsen, D.G. 1981. Studying the biology and control of borers attacking woody plants. Entomological Society of America Bulletin, **27**: 251–259.

Pallardy, S.G. 2008. Physiology of woody plants. 3rd ed. Academic Press, Burlington, Ontario.

Riggins, J.J., Galligan, L.D., and Stephen, F.M. 2009. Rise and fall of red oak borer in the Ozark Mountains of Arkansas. Florida Entomologist, **92**: 428–433. doi:10.1653/024.092.0303.

SAS Institute Inc. 2010. JMP 9.0. SAS Institute Inc., Cary, North Carolina.

Stephen, F.M., Salisbury, V.B., and Oliveria, F.L. 2001. Red oak borer, *Enaphalodes rufulus* (Coleoptera: Cerambycidae), in the Ozark Mountains of Arkansas, U.S.A.: an unexpected and remarkable forest disturbance. Integrated Pest Management Reviews, **6**: 247–252. doi:10.1023/A:1025779520102.

White, T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia, **63**: 90–105. doi:10.1007/BF00379790.

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