The original publication is available at https://www.actahort.org/books/1314/1314_57.htm

Phloem and xylem flow contributions to nectarine fruit development

Riccardo Lo Bianco and Alessio Scalisi
Department of Agricultural, Food and Forest Sciences, University of Palermo, Italy

Abstract
This study aimed at determining how source–sink balance and phloem/xylem flows affect nectarine fruit growth during development. Different levels of water and assimilate availability to growing fruits were induced in vivo by varying leaf:fruit ratio (L:F) of fruiting shoots and by interrupting the phloem stream (girdling) at the base of entire fruiting shoots. Two fruiting shoots in each of six 'Big Top' adult nectarine trees were selected, labeled and their L:F was adjusted by thinning fruits or removing leaves to two levels: high L:F and low L:F. Stem water potential, stomatal conductance, continuous fruit diameter and leaf turgor pressure were measured before and after girdling at stage I and stage III of fruit development. At stage I, fruits from high L:F shoots grew more than those from low L:F shoots both before and after stem girdling. At this stage, xylem flow from the whole tree and phloem flow from leaves of the same stem seem to be the major factors contributing to fruit growth. At stage III, fruits from high and low L:F shoots exhibited similar growth before girdling, whereas girdling induced a generally sharp decline of fruit growth and a marked shrinking of low L:F fruits. At this stage, regular fruit growth mostly relied on both water and carbon phloem imports from neighboring branches. Regardless of girdling or stage, the diel leaf turgor pressure (p_c) range (maximum p_c – minimum p_c over 24 h) was always smaller in leaves from low L:F shoots compared to those from high L:F shoots, indicating that the source-sink balance is a major factor driving water status changes in leaves. Overall, at stage I, nectarine fruit growth was mainly driven by water movements through the xylem, whereas at stage III, it mostly relied on phloem contribution (water + assimilates). Leaf p_c variations were mostly influenced by L:F possibly via xylem backflow from fruits in both stages I and III.

Keywords: fruit gauges, growth stage, leaf turgor, *Prunus persica*, source-sink balance, water potential.

INTRODUCTION

Water and assimilates reach the fruit via the phloem stream and determine fruit growth in terms of both fresh and dry matter. Xylem flows respond to water potential gradients along the vascular path (Minchin and Thorpe, 1987; Minchin et al., 1996). Phloem unloading to the fruit may occur through passive or active mechanisms, depending on whether assimilates reach sink cells via the symplastic route (plasmodesmata network) according to water potential

gradients, or across membranes thanks to specific carbohydrate transporters, respectively (Patrick, 1990; 1997; Lalonde et al., 2003).

Most of the water entering the fruit through the xylem or phloem exits the fruit by epidermal transpiration or, under specific circumstances, via the xylem back to the leaves (xylem backflow). When the total outflow rate exceeds the inflow rate (e.g., at midday during sunny and warm days), fruits shrink, as shown in apple (Lang, 1990), peach (Morandi et al., 2007a), nectarine (Scalisi et al., 2019), kiwifruit (Morandi et al., 2010), pear (Morandi et al., 2014), orange (Lo Bianco et al., 2019), and olive (Scalisi et al., unpublished data). In peach, fruit shrinking during mid to late morning and enlargement during the evening and night has been shown to be more marked during stage III of fruit growth and associated to epidermal permeability and xylem functioning (Morandi et al., 2007a). At this stage, it was suggested that fruit water outflows by transpiration lead to a decrease of fruit water potential and facilitate passive phloem unloading (McFadyen et al., 1996; Patrick, 1997).

Resource availability to growing fruits is affected by crop load, and a condition of source limitation may occur when the source organs are not able to supply all growing sinks, i.e. under high cropping levels (Pavel and Dejong, 1993; Grossman and Dejong, 1995). This may be aggravated by the relatively high degree of branch autonomy reported in peach (Volpe et al., 2008) which may limit the movement of assimilate across branches with different levels of source-to-sink ratios. Pavel and Dejong (1993) found that, in high cropping peach trees, fruit growth was source-limited during stages I and III, and sink-limited during stage II. More recently, Morandi and Corelli Grappadelli (2009) showed that, at certain times of the day, fruits may be more active sinks in attracting resources at high than at low crop loads.

Furthermore, several studies have reported negative effects of high cropping on leaf, stem, and fruit water potentials (Naor et al., 1999; 2001; McFadyen et al., 1996), with possible changes in hydrostatic pressure gradients and direction of the sap flow along the vascular path. All this would affect vascular flows in and out of the fruit, raising the need for more detailed information to understand how water and carbon flows respond to changes in source-to-sink ratios, e.g. leaf-to-fruit ratio of a stem.

In peach, tree vegetative growth (and vigor) has been correlated with tree water status and rootstock hydraulic conductivity (Basile et al., 2003; Solari et al., 2006). If water status is one of the forces driving peach vegetative growth, then vascular flows in and out of the fruit may also be affected by rootstock vigor due to differences in leaf-to-fruit water potential gradients. The present work aimed at determining how source–sink balance and rootstock vigor/tree water status affect nectarine fruit growth at different developmental stages, possibly by changing phloem/xylem flows in and out of the fruit. To pursue this objective, very sensitive precision devices were used to measure continuous variations of fruit diameter and leaf turgor pressure in peach trees grafted on GF677 rootstock and the size-controlling RootPac-40 rootstock (Scalisi et al., 2018).

MATERIAL AND METHODS

The study was conducted in spring 2017 at the Carboj experimental farm

located near Castelvetrano, in southwestern Sicily. Twelve adult 'Big Top' nectarine (*Prunus persica* (L.) Batsch) trees, six grown on high-vigor GF677 (*P. persica* x *P. dulcis*) rootstock and six on low-vigor RootPac-40 [(*P. persica* x *P. persica*) x (*P. dulcis* x *P. persica*)] rootstock at a distance of 5 x 4.5 m and trained to small vase, were selected for the experiment. All trees were exposed to the same conventional management, including drip irrigation and fertilization.

On each tree, two fruiting shoots (45 to 70 cm long) were labeled and used to impose two opposite conditions: one shoot was thinned to two fruits and all leaves were left on (high L:F); the other shoot was left unthinned (1-2 fruits at each node) and partly defoliated (about 30% of leaves removed) (low L:F). At the beginning of the experiment (6 April), trunk circumference (cm) was measured above the graft union with a tape measure and converted into trunk cross-sectional area (TCSA, cm^2). At stages I and III of fruit growth, 28 and 84 days after full bloom (DAFB), respectively, labeled shoots were girdled at the base. All measurements were taken in the two weeks pre- and post-girdling. The entire experiment was carried out at stage I (mid-April to early May, 14-32 DAFB) and stage III of fruit development (early to mid-June, 70-98 DAFB). Before stem girdling, fruits were 8-10 mm in diameter at stage I and 58-60 mm at stage III.

At both stages I and III, before and after stem girdling, leaf stomatal conductance (g_s) was measured during mid-morning using a Delta-T AP4 dynamic porometer (Delta-T Devices LTD, Cambridge, UK) on one sun-exposed leaf per labeled shoot, two per tree. On the same days, a pressure chamber (PMS Instrument Co., Corvallis - Oregon) was used for the determination of midday stem water potential (Ψ_{stem}, at 1200 h). For Ψ_{stem} measurements leaves were covered with plastic wrap and aluminum foil one hour before measurement, as described by Turner (1988).

Fruit gauges described by Morandi et al. (2007b) were used to assess fruit diameter daily fluctuations (FDF), whereas dynamics in leaf turgor pressure (p_c) were determined with leaf patch clamp pressure (LPCP) probes (Yara International, Oslo, NO). Raw data from sensors were smoothed by the Savitzky and Golay (1964) method, using a 15-point convoluted spline and then converted into fruit diameter and p_p. The original output of LPCP probes, namely the attenuated pressure of leaf patches (p_p) in response to clamp pressure, is inversely related to p_c (Zimmermann et al., 2008). Therefore, p_c was estimated using the formula $-1 \times p_p$. FDF and p_c were monitored for four days before and four days after stem girdling at fruit growth stages I and III.

Treatment effects were tested with analysis of variance using Jamovi procedures (Jamovi software, https://www.jamovi.org). Results were plotted using LibreOffice Calc (The Document Foundation, Debian and Ubuntu).

RESULTS AND DISCUSSION

Minor, non-significant differences of Ψ_{stem} in favor of GF677 were only detected at stage I of fruit growth (data not shown). This is in contrast with the association between water status and growth potential shown by Basile et al. (2003). The discrepancy between the two studies may be explained by the fact that our trees in spring were experiencing mild temperatures and high soil

humidity; differences in Ψ_{stem} between the two rootstocks may be more evident during hot and dry days in summer. Due to this lack of significant differences in Ψ_{stem}, data from the two rootstocks were pooled together.

Table 1. Midday stem water potential (MPa) in nectarine shoots under low or high leaf-to-fruit ratio (L:F) before and after girdling at the base of the stem (means ± standard errors). Girdling carried out at 28 and 84 days after full bloom for stage I and stage III, respectively.

Stage I						
	Before			After		
High L:F	-0.40	±	0.037	-0.49	±	0.063
Low L:F	-0.41	±	0.042	-0.47	±	0.062
Stage III						
	Before			After		
High L:F	-0.54	±	0.015	-1.04	±	0.058
Low L:F	-0.53	±	0.044	-1.06	±	0.035

No effect of L:F ratio on Ψ_{stem} was detected in either stage I or III of fruit growth (Table 1). On the other hand, stem girdling significantly reduced Ψ_{stem} only at stage III of fruit growth (Table 1). Similarly there was no effect of L:F ratio on g_s in either stage I or III of fruit growth, just a minor non-significant g_s increase in high L:F probably due to greater fruit sink demand of assimilates.

Table 2. Mid-morning stomatal conductance (mmol m^{-2} s^{-1}) in leaves of nectarine shoots under low or high leaf-to-fruit ratio (L:F) before and after girdling at the base of the stem (means ± standard errors). Girdling carried out at 28 and 84 days after full bloom for stage I and stage III, respectively.

Stage I						
	Before			After		
High L:F	184	±	24.1	185	±	26.9
Low L:F	209	±	15.2	232	±	39.7
Stage III						
	Before			After		
High L:F	392	±	35.9	522	±	101
Low L:F	340	±	32.3	509	±	116

On the contrary, stem girdling showed a more marked effect, increasing g_s mostly at stage III (when fruit sink demand for carbon and water is higher due to its bigger size) and regardless of L:F ratio (Table 2). The latter may be the result of reduced assimilate import from neighboring stems inducing higher photosynthetic rates (and therefore higher g_s) to satisfy fruit sink demand for carbon. In turn, the reductions of Ψ_{stem} observed after phloem girdling may be caused by g_s increases and consequent higher water consumption by transpiration. The lack of L:F ratio effect on g_s during fruit growth suggests that leaves are the major sink for water, especially at stage III of fruit growth when leaf growth is nearly completed.

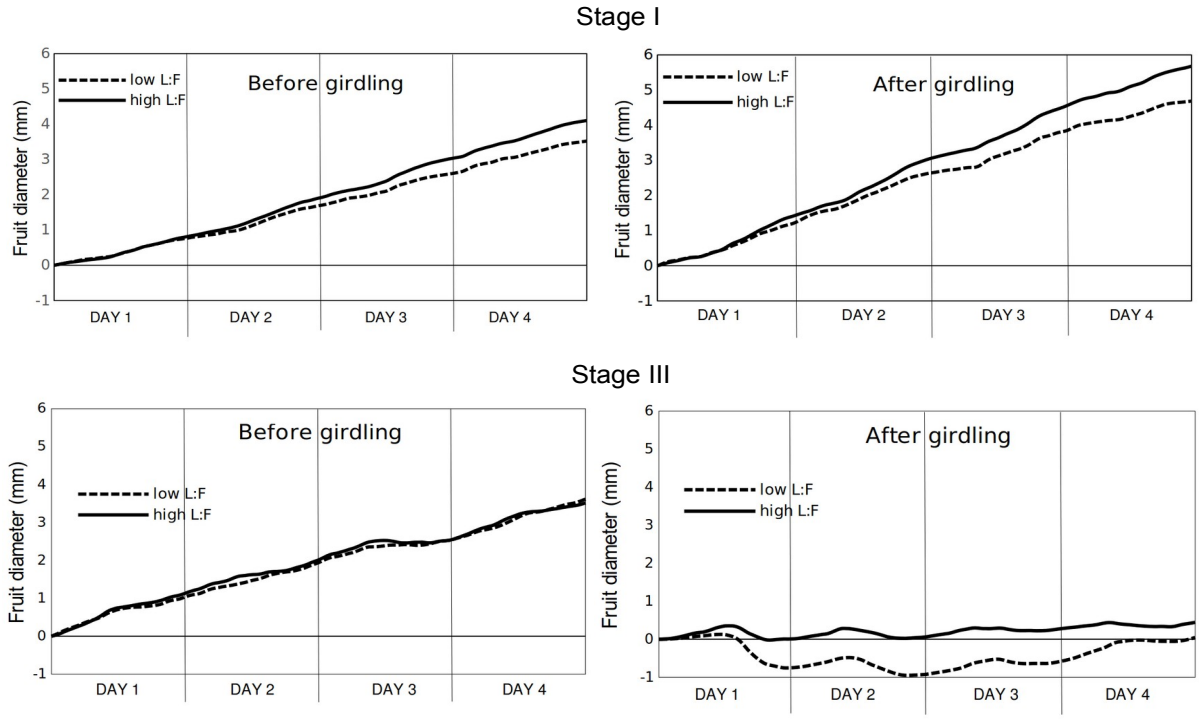

Figure 1. Fruit diameter (mm) over a four-day period in nectarine shoots under low or high leaf-to-fruit ratio (L:F) before (top panels) and after (bottom panels) phloem girdling at the base of the stem (n = 6). Fruits at stage I (24-32 days after full bloom, left panels) and stage III (80-88 days after full bloom, right panels) of development. Girdling carried out at 28 and 84 days after full bloom for stage I and stage III, respectively.

At stage I, fruits from high L:F shoots grew more than those from low L:F shoots, and this difference was slightly more marked after stem girdling (Figure 1). This agrees with previous findings in peach where low L:F ratio reduced fruit growth by affecting carbohydrate dynamics (Morandi et al., 2008). At this stage, xylem flow and phloem flow from leaves of the same stem seem to be the major flows involved in fruit growth, as removal of phloem by girdling did not induce visible changes in growth. At stage III, fruits from high and low L:F

shoots exhibited similar growth before girdling (Figure 1). At this stage, phloem disruption by girdling induced a generally sharp decline of fruit growth and a marked shrinking of low L:F fruits, indicating that, under these specific conditions, regular fruit growth relied on both water and carbon phloem imports from neighboring branches. This agrees substantially with previous observations of carbon labeling studies showing that extreme source-to-sink imbalances are needed for carbon to move across different stems and branches (Volpe et al., 2008).

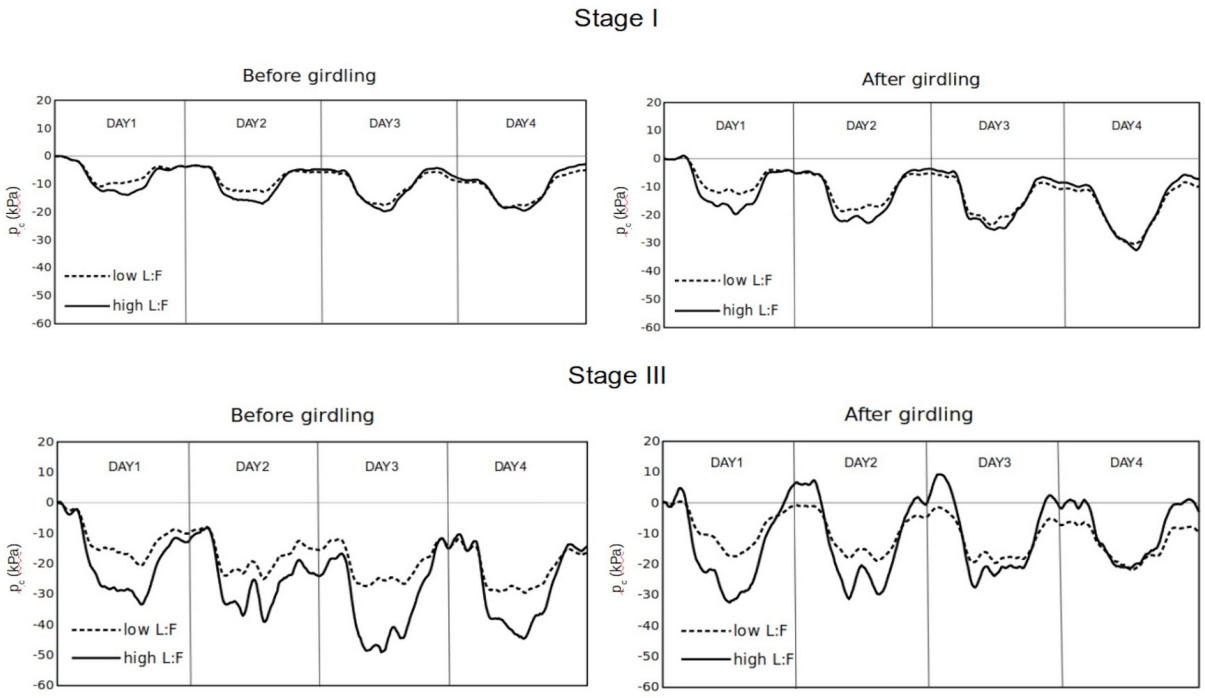

Figure 2. Leaf turgor pressure (p_c, starting at 0 kPa) over a four-day period in leaves (n = 6) of nectarine shoots under low or high leaf-to-fruit ratio (L:F) before (top panels) and after (bottom panels) phloem girdling at the base of the stem. Fruits at stage I (left panels) and stage III (right panels) of development. Girdling carried out at 28 and 84 days after full bloom for stage I and stage III, respectively.

Regardless of fruit growth stage, leaf to fruit ratio and girdling, leaves gradually lost some turgor pressure, mainly because of gradual soil drying between irrigation cycles (Figure 2). Yet, the diel p_c range (maximum – minimum p_c over 24 h) was always greater in leaves from high L:F shoots compared to those from low L:F shoots (Figure 2), indicating that the source-sink balance is a major factor driving water status changes in leaves. On the one hand, in shoots with fewer leaves and many fruits, xylem backflow from fruits may have significantly contributed to contain p_c fluctuations due to leaf transpiration; on the other hand, in shoots with a low number of fruits and numerous leaves, the total leaf transpiring area was greater and fruits were not able to contain p_c

fluctuations by backflow. This difference was particularly marked at stage III, when leaves were transpiring more due to higher air temperatures (see also g_s levels in Table 2), and even more after girdling (Figure 2). The latter suggests that stem girdling at this stage interrupted phloem flow from neighbor branches toward fruiting shoots, reducing the external supply of carbon and water and, in turn, the contribution of fruits to leaf water balance.

CONCLUSIONS

At stage I, nectarine fruit growth is mainly driven by water movements through xylem, whereas at stage III, it also relies on phloem contribution (water + assimilates) from neighboring shoots. Leaf p_c variations are mostly dependent on xylem activity and phloem vascular flows to nearby organs, and they are significantly affected by L:F, suggesting a determinant role of fruit water backflow for leaf water balance.

Overall, this experiment confirmed what seen in previous studies for assimilate allocation within the single stem and across stems, although it indicated that also water movement across stems through the phloem may be an important factor for fruit growth and leaf gas exchange under source limiting conditions.

Literature cited

Basile, B., Marsal, J., Solari, L.I., Tyree, M.T., Bryla, D.R., and DeJong, T.M. (2003). Hydraulic conductance of peach trees grafted on rootstocks with differing size-controlling potentials. J. Hortic. Sci. Biotechnol. *78*, 768–774.

Grossman, Y.L. and Dejong, T.M. (1995). Maximum fruit growth potential and seasonal patterns of resource dynamics during peach growth. Ann. Bot. *75*, 553–560.

Lalonde, D., Tegeder, M., Throne-Holst, M., Frommer, W.B., and Patrick, J.W. (2003). Phloem loading and unloading of sugars and amino acids. Plant Cell Environ. *26*, 37–53.

Lang, A. (1990). Xylem, phloem and transpiration flows in developing apple fruits. J. Exp. Bot. *41*, 645–651.

Lo Bianco, R., Grilo, F.S., Scalisi, A., Pernice, F., and Morandi, B. (2019). Recurrent deficit irrigation and fruit harvest affect tree water relations and fruitlet growth in 'Valencia' orange. Europ. J. Hort. Sci. *84*(3), 177–187.

McFadyen, L.M., Hutton, R.J. and Barlow, E.W.R. (1996). Effects of crop load in fruit water relations and fruit growth in peach. J. Hortic. Sci. *71*, 469–480.

Minchin, P.E.H. and Thorpe, M.R. (1987). Is phloem transport due to a hydrostatic pressure gradient? Supporting evidence from pressure chamber experiments. Aust. J. Plant Physiol. *14*, 397–402.

Minchin, P.E.H., Thorpe, M.R., Hall, J.L., Baker, D.A., and Oparka, K.L. (1996). What determines carbon partitioning between competing sinks? J. Exp. Bot. *47*, 1293–1296.

Morandi, B., Rieger, M.W., and Corelli Grappadelli, L. (2007a). Vascular flows and transpiration affect peach (*Prunus persica* Batsch.) fruit daily growth. J. Exp. Bot. *58*, 3941–3947.

Morandi, B., Manfrini, L., Zibordi, M., Noferini, M., Fiori, G., and Corelli Grappadelli, L. (2007b). A low-cost device for accurate and continuous measurements of fruit diameter. HortScience *42*(6), 1380-1382.

Morandi, B., Corelli Grappadelli, L., Rieger, M.W., and Lo Bianco, R. (2008). Carbohydrate availability affects growth and metabolism in peach fruit. Physiol. Plant. *133*, 229–241.

Morandi, B. and Corelli Grappadelli, L. (2009). Source and sink limitations in vascular flows in peach fruit. J. Hortic. Sci. Biotechnol. *ISAFRUIT S. I.* 150–156.

Morandi, B, Manfrini, L., Losciale, P., Zibordi, M., and Corelli Grappadelli, L. (2010). Changes in vascular and transpiration flows affect the seasonal and daily growth of kiwifruit (*Actinidia deliciosa*) berry. Ann. Bot. *105*, 913–923.

Morandi, B., Losciale, P., Manfrini, L., Zibordi, M., Anconelli, S., Pierpaoli, E., and Corelli Grappadelli, L. (2014). Leaf gas exchanges and water relations affect the daily patterns of fruit growth and vascular flows in Abbé Fétel pear (*Pyrus communis* L.) trees. Sci. Hortic. *178*, 106–113.

Naor, A., Klein, I., Hupert, H., Grinblat, Y., Peres, M. and Kauffman, A. (1999). Water stress and crop level interactions in relation to nectarine yield, fruit size distribution and water potentials. J. Am. Soc. Hortic. Sci. *124*, 189–193.

Patrick, J.W. (1990). Sieve element unloading: cellular pathway, mechanism and control. Physiol. Plant. *78*, 298–308.

Patrick, J.W. (1997) Phloem unloading: sieve element unloading and post-sieve element transport. Annu. Rev. Plant Physiol. Plant Mol. Biol. *48*, 191–222.

Pavel, E.W. and Dejong, T.M. (1993). Source- and sink-limited growth periods of developing peach fruits indicated by relative growth rate analysis. J. Am. Soc. Hortic. Sci. *118*, 820–824.

Scalisi, A. Lo Bianco, R., Caruso, T., Giovannini, D., Sirri, S., and Fontana, F. (2018). Preliminary evaluation of six *Prunus* rootstocks for peach in Italy. Acta Hortic. 1228, 273-278.

Scalisi, A., O'Connell, M., Stefanelli, D., Lo Bianco, R. (2019). Fruit and leaf sensing for continuous detection of nectarine water status. Frontiers in Plant Sci. *10*, 805.

Solari, L.I., Johnson, S., and DeJong, T.M. (2006). Relationship of water status to vegetative growth and leaf gas exchange of peach (*Prunus persica*) trees on different rootstocks. Tree Physiol. *26* (10), 1333-1341.

Turner, N.C. (1988). Measurement of plant water status by the pressure chamber technique. Irrig. Sci. *9* (4), 289-308.

Volpe, G., Lo Bianco, R., and Rieger, M. (2008). Carbon autonomy of peach shoots determined by ^{13}C-photoassimilate transport. Tree Physiol. *28*, 1805-1812.

Zimmermann, D., Reuss, R., Westhoff, M., Gessner, P., Bauer, W., Bamberg, E., Bentrup, F.-W., and Zimmermann, U. (2008). A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. J. Exp. Bot. *59* (11), 3157–3167.