Corrections

PLANT BIOLOGY

The authors note that Fig. 3 appeared incorrectly. The corrected figure and its legend appear below. This error does not affect the conclusions of the article.

Fig. 3. Auxin levels and signaling are enhanced in max1 mutants. (A) In pin1-613 and pin3-5 mutants, the acropetal progression of IC initiation is diminished. Plants were analyzed when shoots were 2, 5, 15, and 30 cm tall. (B) Comparison of levels of free IAA in wild type and max1-1 at different positions along the inflorescence stem. The first elongated internode above the rosette was counted as the first internode. IN, internode. (C and D) Analysis of DR5:GUS activity in wild-type (C) and max1-1 inflorescence stems (D). Rosette leaves have been removed for clarity. (E–H) Analysis of DR5rev:GFP activity at different positions of the inflorescence stem. (E and F) DR5rev:GFP detection 1 cm above the rosette in wild type (E) and max1-1 (F). (G and H) DR5rev:GFP detection immediately above the uppermost rosette leaf of wild type (G) and max1-1 (H). [Scale bar in D (5 mm) also applies to C; scale bar in E (100 μm) also applies to F–H.]
ECOLOGY, STATISTICS

The authors note that the data for three of the eight datasets analyzed in the article (Skipwith, St. Martin, and Ythan) were incorrectly processed. As a result, Fig. 2, Table 1, and Table 2 appeared incorrectly. The corrected figure, its corrected corresponding legend, and the corrected tables appear below. These errors do not affect the conclusions of the article.

Fig. 2. Food web graphs displaying trophic structure from fitting model 1 with phylogeny measure $x_j$ as the predictor. (SR) Feeding activity in Bay. The $s$ axis refers to activity as prey, and the $r$ axis, as predator. Label of node $i$ is located at the mean of the bivariate posterior distribution (an "estimate") of $[s_i, r_i]$; this distribution describes the probability of the position of $[s_i, r_i]$ on the $sr$ plane, given the knowledge of the observed food web. Distribution density appears as heat map for benthos-eating birds ("37") and detritus ("48"). Legend for node labels appears in SI Text. (U, V) Preference of being consumed/consuming in Skipwith. They are similarly interpreted as (SR), but referring to $u_i$ vectors for small oligochaetes ("2"), C. praeusta ("14"), L. marmoratus ("30"), and detritus ("37"), and $v_i$ vectors for A. juncea ("11"), A. germari ("19"), great diving beetle ("27"), and P. sagittalis ("31"). Nodes far apart in the latent $u$ or $v$ space differ substantially with respect to feeding preference.
Table 1. Bayesian inference numerical summaries for selected food webs from fitting statistical social network model 1, with phylogenetic similarity $x_{ij}$ as the predictor

<table>
<thead>
<tr>
<th>Food web*</th>
<th>Parameter</th>
<th>Posterior median†</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Credibility‡</th>
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<tbody>
<tr>
<td>Bay</td>
<td>$\beta_1$</td>
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<td>1.20</td>
<td>10.46</td>
<td>0.95</td>
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<tr>
<td></td>
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<td></td>
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<tr>
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<tr>
<td></td>
<td>$\rho_{sr}$</td>
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<td>(interval includes 0)</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\rho$</td>
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<td>-0.02</td>
<td>0.60</td>
</tr>
<tr>
<td>Skipwith</td>
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<td>(interval includes 0)</td>
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</tr>
<tr>
<td></td>
<td>$\rho_{sr}$</td>
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<td></td>
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<td>St. Martin</td>
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<td></td>
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<td>$\rho$</td>
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<td>(interval includes 0)</td>
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*Other food webs and $z_{ij}$ appear in Table S2.

†The posterior median can be considered a parameter “estimate.”

‡Credible intervals presented have approximately the highest credibility without including 0. High credibility for an interval excluding 0 indicates statistical importance of the corresponding parameter to feeding potential ($p_{ij}$).

Table 2. Goodness-of-fit summaries for selected food webs (others in Table S2)

<table>
<thead>
<tr>
<th>Model</th>
<th>Predator</th>
<th>GoF*</th>
<th>Model</th>
<th>Predictor</th>
<th>GoF</th>
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<td>1</td>
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<td>$z_{ij}$</td>
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<tr>
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<td>—</td>
<td>394</td>
<td>1</td>
<td>—</td>
<td>533</td>
</tr>
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<td>$x_{ij}$</td>
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</tr>
<tr>
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<td>$z_{ij}$</td>
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<td>naive</td>
<td>$z_{ij}$</td>
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<tr>
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<td>1</td>
<td>$z_{ij}$</td>
<td>275</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
<td>33</td>
<td>1</td>
<td>—</td>
<td>286</td>
</tr>
<tr>
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<tr>
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<td>$z_{ij}$</td>
<td>737</td>
<td>naive</td>
<td>$z_{ij}$</td>
<td>678</td>
</tr>
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</table>

*Derived from the Bayes factor on the model’s ability to predict the act of feeding ($p_{ij}$). When comparing between models, noticeably smaller GoF values suggest better fit.

†Simple logistic regression ignoring network dependence—i.e., naively setting $s_i + r_j + u_i z_{ij} + c_{ij} = 0$ for all $i,j$ in model 1.
Strigolactone signaling is required for auxin-dependent stimulation of secondary growth in plants

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Long distance cell-to-cell communication is critical for the development of multicellular organisms. In this respect, plants are especially demanding as they constantly integrate environmental inputs to adjust growth processes to different conditions. One example is thickening of shoots and roots, also designated as secondary growth. Secondary growth is mediated by the vascular cambium, a stem cell-like tissue whose cell-proliferating activity is regulated over a long distance by the plant hormone auxin. How auxin signaling is integrated at the level of cambium cells and how cambium activity is coordinated with other growth processes are largely unknown. Here, we provide physiological, genetic, and pharmacological evidence that strigolactones (SLs), a group of plant hormones recently described to be involved in the repression of shoot branching, positively regulate cambial activity and that this function is conserved among species. We show that SL signaling in the vascular cambium itself is sufficient for cambium stimulation and that it interacts strongly with the auxin signaling pathway. Our results provide a model of how auxin-based long-distance signaling is translated into cambium activity and suggest that SLs act as general modulators of plant growth forms linking the control of shoot branching with the thickening of stems and roots.

Hormone-based long-distance signaling is essential for coordinating the growth and activity of different organs and tissues during the life cycle of multicellular organisms. In particular, plants are demanding in this respect because, due to their sessile and indeterminate lifestyle, their reproductive success depends on the competence to adjust their developmental programs to changing environmental conditions and requirements. Auxin is one of the best-characterized hormones involved in long-distance signaling, regulating a tremendous number of developmental processes. One of these is apical dominance, which, indeed, represents a classical example for long-distance signaling in plants (1). In this case, apex-derived auxin is transported basipetally along the main shoot and this is required for suppressing the outgrowth of axillary buds. Another process that depends on the basipetal transport of auxin is secondary growth (2). Secondary or lateral growth of growth axes is based on the activity of the vascular cambium, a meristematic tissue organized as a cylinder encompassing the center of growth axes. Cambium activity leads to the production of secondary vascular tissue, which results in an increase of shoot or root diameter. The interconnectedness of the process by decapitation of the shoot tip and the subsequent reversibility by artificial auxin application is shared between apical dominance and secondary growth (1, 2). Even though both processes thus share upstream processes and, presumably, regulators, how basipetal transport of auxin is translated into two different outputs and to what degree they are interconnected is unknown.

Significant progress has been made in the understanding of the molecular control of apical dominance by the characterization of the strigolactone (SL) biosynthesis and signaling pathway. SLs, a carotenoid-derived group of molecules, show all attributes of plant hormones, meaning that they travel through the plant (3) and are effective at low concentrations (4, 5). In addition to shoot branching, SLs have recently been identified as germination stimulants of parasitic plants and as triggers for the establishment of interactions with mycorrhizal fungi, in which context they were originally identified (reviewed in ref. 6). The SL signaling pathway has, so far, been defined by a series of four genes for which homologs have been characterized in Arabidopsis (7–10), pea (11), petunia (12), tomato (13), and rice (14). Also in rice, two additional genes associated with the SL pathway have been recently described, indicating that the discovery of SL-related genes is not yet completed (15, 16). The identification of SL signaling in distantly related species, among them mono- and dicotyledonous species, suggests that this communication tool represents an ancient invention and that it is widely distributed within the plant kingdom.

The function of SL-related genes is characterized best in Arabidopsis where three of four more AXILLARY BRANCHES (MAX) proteins (MAX1, MAX3, and MAX4) have been suggested to be involved in the biosynthesis of SLs starting from carotenoids. This suggestion is based on their resemblance to carotenoid dioxygenases (MAX3 and -4) and to cytochrome P450 family members (MAX1), on corresponding biochemical activities (7–9) and on SL deficiency of knockout mutants (4, 5, 17). In contrast to max1, -3, and -4 mutants, max2 mutants are SL insensitive with regard to an effect on apical dominance (4, 5, 9), consistent with the idea that MAX2 functions as a receptor for SLs or an unknown downstream product. Moreover, its similarity to F-box leucin-rich repeat proteins and its physical interaction with compounds of Skp/Cullin/F-box (SCF) E3 ligase complexes suggests that MAX2 functions as an SL-dependent recruiting factor for proteins destined for proteasomal degradation (10).

max2 mutants (which were also isolated as ore9) (18) display various growth alterations in addition to a decrease of apical dominance, like delayed leaf senescence (18, 19), altered leaf...
morphology (19), and hypocotyl elongation (20). In addition, the MAX2 gene is required for karrikin-induced seed germination in postfire environments (21) and a role of the SL signaling pathway in the regulation of root system architecture was also described recently (17, 22, 23). Intriguingly, in contrast to pea and rice (4, 24), SLs are only reduced by 50% in Arabidopsis max1 and max4 mutants (17). Together, these observations suggest that the broadness of SL-dependent processes is currently underestimated.

One explanation for a broader role of the SL signaling pathway in various developmental processes is an interactive mode of action with the auxin signaling pathway. The interaction between both pathways seems to exist on several levels. On the one hand, the influence of SL signaling on auxin transport has been reported. max mutants in Arabidopsis display an enhanced expression of PIN1 and PIN3, two members of the auxin efflux transporter family and show an increase in auxin transport capacity (25–29). These observations indicate that SLs act indirectly on apical dominance by repressing auxin transport, which impedes auxin synthesized in lateral buds to be transported into the main stem (25, 29, 30). On the other hand, SL-dependent inhibition of the outgrowth of side shoots functions also in auxin-depleted plants and the application of SLs and auxin transport inhibitors, like N-1-naphthylphthalamic acid (NPA), results in different physiological responses (26). This suggests SL’s function to also be downstream of auxin signaling. In fact, MAX4 and RMS1, its homolog in pea, are inducible by auxin application (7, 31), and the same holds true for RMS5, the pea homolog of MAX3 (32). Even though the significance of MAX4 inducibility has been questioned (33), these observations indicate that SL biosynthesis is stimulated by auxin.

In this study, we reveal a role of SL signaling in the regulation of secondary growth and show that the regulation of cambium activity is an SL response, which is independent from the regulation of apical dominance and conserved among species. On the basis of genetic and pharmacological data, we propose that SLs fulfill their roles directly in the cambium mainly downstream of auxin signaling.

Results

**Strigolactone-Deficient Mutants Display a Reduction in Secondary Growth.** To characterize long-distance regulation of the cambium, we studied the effect of SLs on cambium activity in wild-type and max mutant Arabidopsis plants. Cambium activity was defined by the lateral extension of the tissue produced by the interfascicular cambium (IC) immediately above the uppermost rosette leaf (IC-derived tissue; ICD) and by the acropetal progression of IC initiation along the inflorescence stem (34, 35). Quantifying these parameters, we observed that the IC-based tissue production was decreased by 30% in all max mutants (Fig. A–D) and that the progression of IC initiation was reduced on average by 40% (Fig. 1F). Consistent with a reduced cambium activity in SL-defective plants, qRT-PCR analyses demonstrated lower transcript levels of cambium-specific (35) and cell cycle-related (36, 37) genes in max1-1 stems in comparison with wild type (Fig. S1). Together, these observations demonstrate that plants with reduced SL signaling or biosynthesis show reduced cambium activity.

**Local GR24 Treatments Stimulate Cambium Activity.** To test whether the reduction in cambium activity in max mutants is based on a direct regulation of the vascular cambium by SLs, we treated wild-type, max1-1, and max2-1 stems locally with the synthetic SL analog GR24. Stems were treated and analyzed in the first internode of the main inflorescence stem in a region where, without treatment, no IC is established in any of the genotypes used in this study (Fig. 1G) (34). In contrast to mock treatments, locally applied GR24 induced cell divisions in interfascicular regions of plants of all three genetic backgrounds at the treatment site (Fig. 2 A–C). The pattern of cell divisions was similar to that observed during secondary growth initiation at the base of the inflorescence stem of untreated plants (Fig. 1A and B) (34), and the effect was dose dependent (Fig. S2A). max1-1 mutants were more sensitive than wild type and, consistent with the employment of the canonical SL signaling pathway, the effect was significantly weaker in max2-1 (Fig. 2C). Remarkably, max2-1

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** Genetic analysis of the role of SL signaling and biosynthesis in cambium regulation. (A–D) Cross-sections from immediately above the uppermost rosette leaf of wild-type (A and B) and max1-1 (C and D) stems. Note that B and D are higher magnification images for areas labeled in A and C, respectively. The extension of the IC-derived tissue (ICD) is indicated by braces (I). Scale bar in C (100 μm) also applies to A, and scale bar in D (50 μm) also applies to B. Asterisks indicate the position of primary vascular bundles. (E) Quantification of lateral ICD extension immediately above the uppermost rosette leaf as indicated in A and C. (F) Quantification of the longitudinal progression of IC initiation, as illustrated in D. (G) Scheme of longitudinal IC extension in the Arabidopsis stem (red) and the relative position of the treatment zone described in Fig. 2 and Fig. 4.
Initially only active in the phloem of primary bundles, an APL::CFP reporter was also active in clusters of cells in interfascicular regions in wild-type backgrounds (25, 28). Because auxin is a positive key regulator of cambium activity (35, 41, 42), we thus analyzed whether SLs stimulate cambium activity indirectly by repressing auxin transport, which might result in enhanced auxin accumulation in certain cell types. Initially, we analyzed pin1-613 and pin3-5 mutants to see how changes in auxin transport capacities influence cambium activity. Both mutants were chosen because PIN1 and PIN3 are strongly expressed in stems (Fig. S2C) and, at least for pin1 mutants, it has been shown that auxin transport capacity along the stem is strongly impaired (43). Histological analysis of the acropetal progression of IC initiation and ICD extension revealed a decrease in both pin mutants (Fig. S4 and Fig. S3), implying that basipetal auxin transport along the stem is positively correlated with cambium activity and that an enhanced auxin transport rate in max mutants is not causal for decreased cambium activity.

Next, we measured the concentration of free indole-3-acetic acid (IAA) in the stems of wild-type and max1-1 mutants at different positions including the segment immediately above the uppermost rosette leaf displaying pronounced cambium activity (34). Our analyses revealed enhanced IAA concentration in max1-1 stems at all positions with an increasing difference toward the shoot base, where max1-1 contained ~5.5 times more IAA in comparison with wild type (Fig. 3B). To determine whether enhanced IAA concentration also leads to enhanced auxin signaling, especially in segments with prominent secondary growth, we analyzed DR5::GUS reporter activity visualizing auxin signaling (44) in stems of both wild-type and max1-1 mutants. Enhanced reporter gene activity was detected in max1-1 along the whole stem including the base, where secondary growth is most prominent (34) (Fig. 3 C and D). We also analyzed the distribution of auxin signaling at tissue level resolution in wild-type and max1-1 backgrounds taking advantage of the DR5rev::GFP reporter (45). The analysis of stem cross-sections revealed a comparable pattern of reporter gene activity with more intense activity in max1-1 than in wild type. Increased reporter gene activity was observed in tissues including primary bundles along the whole stem and in interfascicular regions at the stem base where IC formation takes place (Fig. 3 E–H).

Collectively, these results show that, even though secondary growth is reduced, auxin concentration and signaling is enhanced in the vasculature of plants with reduced SL biosynthesis. This suggests that the decrease in secondary growth observed in max

plants were not fully GR24 insensitive with respect to the induction of cambium-like cell divisions, which is in contrast to their insensitivity with respect to other processes (5, 23) and suggests that there are factors acting in parallel to MAX2 in this case. Collectively, these observations show that GR24 can locally stimulate cambium-like cell divisions in the Arabidopsis inflorescence stem.

Next, we tested whether GR24-induced cell divisions represent secondary growth as observed under natural conditions by analyzing the dynamics of cambium- and phloem-specific markers in response to GR24 treatments. PHLOEM INTERCALATED WITH XYLEM (PXY, also known as TDIF RECEPTOR, TDR) is a receptor-like kinase expressed in cambium cells throughout the Arabidopsis plant body (38, 39). A reporter line expressing the cyan fluorescent protein (CFP) under the control of the PXY promoter (PXY::CFP) visualized the fascicular cambium in primary stems and, after GR24 treatments, was detected in interfascicular regions indicating GR24-induced formation of the interfascicular cambium (Fig. 2 D and E). To visualize the formation of secondary vascular tissue, we used the promoter of the APL gene encoding for a MYELOBLASTOSIS (MYB) transcription factor specifically expressed in phloem tissues (40).

Increased Auxin Content and Signaling in SL Mutant Inflorescence Stems. Previous reports showed that SL signaling can reduce auxin transport in the Arabidopsis stem (25–27, 29), presumably by suppressing members of the PIN family of auxin transporters (25–27, 29). Furthermore, the analysis of auxin-sensitive reporters and measurements of auxin exported out of isolated stem fragments suggest that auxin levels are increased in max mutant backgrounds (25, 28). Because auxin is a positive key regulator of cambium activity (35, 41, 42), we thus analyzed whether SLs stimulate cambium activity indirectly by repressing auxin transport, which might result in enhanced auxin accumulation in certain cell types. Initially, we analyzed pin1-613 and pin3-5 mutants to see how changes in auxin transport capacities influence cambium activity. Both mutants were chosen because PIN1 and PIN3 are strongly expressed in stems (Fig. S2C) and, at least for pin1 mutants, it has been shown that auxin transport capacity along the stem is strongly impaired (43). Histological analysis of the acropetal progression of IC initiation and ICD extension revealed a decrease in both pin mutants (Fig. S4 and Fig. S3), implying that basipetal auxin transport along the stem is positively correlated with cambium activity and that an enhanced auxin transport rate in max mutants is not causal for decreased cambium activity.

Next, we measured the concentration of free indole-3-acetic acid (IAA) in the stems of wild-type and max1-1 mutants at different positions including the segment immediately above the uppermost rosette leaf displaying pronounced cambium activity (34). Our analyses revealed enhanced IAA concentration in max1-1 stems at all positions with an increasing difference toward the shoot base, where max1-1 contained ~5.5 times more IAA in comparison with wild type (Fig. 3B). To determine whether enhanced IAA concentration also leads to enhanced auxin signaling, especially in segments with prominent secondary growth, we analyzed DR5::GUS reporter activity visualizing auxin signaling (44) in stems of both wild-type and max1-1 mutants. Enhanced reporter gene activity was detected in max1-1 along the whole stem including the base, where secondary growth is most prominent (34) (Fig. 3 C and D). We also analyzed the distribution of auxin signaling at tissue level resolution in wild-type and max1-1 backgrounds taking advantage of the DR5rev::GFP reporter (45). The analysis of stem cross-sections revealed a comparable pattern of reporter gene activity with more intense activity in max1-1 than in wild type. Increased reporter gene activity was observed in tissues including primary bundles along the whole stem and in interfascicular regions at the stem base where IC formation takes place (Fig. 3 E–H).

Collectively, these results show that, even though secondary growth is reduced, auxin concentration and signaling is enhanced in the vasculature of plants with reduced SL biosynthesis. This suggests that the decrease in secondary growth observed in max

Figure 2. GR24 treatments stimulate secondary growth in Arabidopsis inflorescence stems. (A and B) Sections from GR24- (A) and mock-treated (B) wild-type plants. Extension of tissue produced in interfascicular regions is indicated by the brace (i) in A. (C) Quantification of GR24-induced tissue production in interfascicular regions of wild-type, max1-1, and max2-1. Note that in mock-treated plants no cell divisions were observed in any of the genetic backgrounds (shown here for wild type). (D and E) PXY::CFP activity in mock- (D) and GR24-treated plants (E). Reporter gene-derived signal in the fascicular cambium is indicated by arrows in D and in interfascicular regions in E. (F and G) APL::CFP activity in mock- (F) and GR24-treated plants (G). Reporter gene-derived signal in the phloem of vascular bundles is indicated by arrows in F and in interfascicular regions in G. (H) APL::GUS activity in GR24- (Left) and mock-treated (Right) plants. (G) PXY::GUS detection in GR24- (Left) and mock-treated (Right) plants. Asterisks indicate the position of vascular bundles. [Scale bar in B (100 μm)] also applies to A; scale bar in D (100 μm) also applies to E–G; scale bar in I (5 mm) also applies to H.] Note that sections in G–I were counterstained by propidium iodide (red), which highlights cell walls.
Local treatments of stems by NPA are able to block mutants, the acropetal progression of IC initiation is DR5rev:GFP mutants. max local NPA treatments of wild-(100 D under the control of the mutation should not reduce mutants (| stems. We observed that interfa- (40), and activity can be found in a broad mutants are impaired in auxin PNAX mutans are not MAX2 vol. 108 and (max promoters, respectively. RT-PCR with primers speci- (pro)cambium-speci- cambium activity, then interaction of auxin and SL signaling pathways. We speculated that if SL signaling is important for a positive effect of auxin on cambium activity, then max mutants should exhibit either no response or, at least, a weaker response than wild type. To test this hypothesis, we performed local NPA treatments of wild-type, max1-1, and max2-1 stems. We observed that interfascicular tissue production in NPA-treated max1-1 and max2-1 mutants was reduced by 75% (Fig. 4A), indicating that SL biosynthesis and signaling is important for auxin to stimulate vascular cambium activity. The fact that max mutants are not completely devoid of cambium activity might be explained by an SL-independent effect of auxin on the cambium or alternatively, by redundancy in the MAX pathway or a role of as yet unidentified factors.

To determine whether SL and auxin signaling might act sequentially or in parallel, we tested the genetic interaction between the axr1-3 and max mutants. axr1-3 mutants are impaired in auxin signaling (47) and we reasoned that mutant phenotypes should not be additive if both act through a common signaling pathway. Consistent with a role of AXR1 in cambium regulation, IC initia- tion and activity was reduced in axr1-3 mutants (Fig. S4). As expected, axr1-3 max double mutants displayed the same reduction in the NPA response as the corresponding single mutants (Fig. 4B). These findings support a sequential order of the two signaling pathways and, together with the observation of elevated auxin levels in max1-1 mutants and their reduced NPA responsiveness, suggest that SLs act downstream of auxin in a common signaling cascade. If this is the case, the axr1-3 mutation should not reduce GR24 responsiveness. Indeed, histological analyses showed that axr1-3, axr1-3 max1-1, and axr1-3 max2-1 plants displayed the same GR24-induced response as wild type, max1-1, and max2-1, respectively (Fig. 4B). Taken together, these results indicate that SLs function predominantly downstream of auxin in a signaling cascade that positively regulates secondary growth.

**Tissue-Specific SL Signaling.** MAX2 activity can be found in a broad spectrum of tissues (10) and the other MAX genes can influence shoot branching over long distances (3). To dissect whether SL signaling in the vascular cambium is sufficient for stimulating cambium activity, we expressed MAX2 under the control of the (pro)cambium-specific WOX4 promoter (48) in max2-1 mutants. As controls, we also established lines expressing MAX2 in the starch sheath, phloem, and xylem, with the help of the SCR (49), APL (40), and NST3 (50) promoters, respectively. RT-PCR with primers specific for the respective constructs demonstrated that all transgenes were actively transcribed (Fig. S2D). We analyzed the lateral ICD extension at the base of the main inflorescence stem from two independent lines for each construct. This analysis demonstrated that only the lines expressing MAX2 in the vascular cambium displayed secondary growth at wild-type-like levels (Fig. 4C). Lines expressing MAX2 within the phloem or the starch sheath displayed no difference compared with max2-1, whereas only a minor increase could be observed in lines expressing MAX2 in the xylem (Fig. 4C). All in all, our results show that SL signaling within the vascular cambium is sufficient to promote cambium activity arguing that MAX2-dependent SL signaling regulates the process in a cell-autonomous manner.

**SL-Dependent Cambium Regulation Is Conserved Between Species.** To elucidate whether SLs fulfill a positive role in cambium regulation in woody species displaying prominent secondary growth, we performed local GR24 treatments on stems of Eucalyptus globulus. As a result, we observed a significant increase in the production of secondary vascular tissues and in particular, the lateral extension of the cambium zone was increased (Fig. S5). To see whether the endogenous SL pathway itself is involved in cambium regulation in species other than Arabidopsis, we quanti- fied cambium activity in the pea mutant ms1-1, impaired in the activity of the MAX4 ortholog (7, 32). Our analysis revealed a significant reduction in cambium activity in comparison with wild type (Fig. S6). Collectively, these results suggest a conserved role for SLs in the regulation of cambium activity across species, including species with prominent wood production.

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**Fig. 3.** Auxin levels and signaling are enhanced in max1 mutants. (A) In pin1-613 and pin3-5 mutants, the acropetal progression of IC initiation is diminished. Plants were analyzed when shoots were 2, 5, 15, and 30 cm tall. (B) Comparison of levels of free IAA in wild type and max1-1 at different positions along the inflorescence stem. The first elongated internode above the rosette was counted as the first internode. th, internode. (C and D) Analysis of DRS5:GUS activity in wild-type (C) and max1-1 inflorescence stems (D). Rosette leaves have been removed for clarity. (E–H) Analysis of DRS5rev: GFP activity at different positions of the inflorescence stem. (E and F) DRS5rev: GFP detection 1 cm above the rosette in wild type (E) and max1-1 (F). (G and H) DRS5rev: GFP detection immediately above the uppermost rosette leaf of wild type (G) and max1-1 (H). (Scale bar in D (5 mm) also applies to C; scale bar in E (100 μm) also applies to F–H.)
mutant stems and that axr1-3 max1-1
and max mutants. Third, genetic
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toward NPA is, at least partly, due to a reduced SL bio-
axr1-3 AXR1 SCR:MAX2 and axr1-3 like levels of cambium activity (Fig. 4),
and show that SL responsiveness is not impaired in auxin sig-
SL signaling for auxin becoming fully active on cambium cells
analyses of the interaction between SL and auxin signaling in the
context of cambium regulation are in line with a requirement of
interfascicular regions of wild type, max1-1, max2-1, axr1-3, axr1-3 max1-1, and axr1-3 max2-1. Note that in mock-treated plants no interfascicular cell
divisions were observed in any of the genetic backgrounds (shown here for wild type). (C) Quantification of lateral ICD extension immediately above the
uppermost rosette leaf in wild-type, max2-1, and max2-1 plants carrying APL:MAX2, NST3:MAX2, SCR:MAX2, or WOX4:MAX2 transgenes. For each construct,
two independent transgenic lines were analyzed. In all cases, n = 10.

Discussion
In this work, we present the role of SLs and their interaction with
the auxin signaling pathway in the control of secondary growth. We report a significant reduction in cambium activity in SL-de-
cicient mutants (Fig. 1) and show induction of secondary growth
upon local treatments with the SL analog GR24 (Figs. 1 and 2). Collectively, these results demonstrate a positive role of SL
signaling in the regulation of cambium activity.
Previous studies have shown that PIN protein levels and auxin
transport capacity are enhanced in max1-1 mutant stems and that
GR24 applications reduce PIN1 protein levels and dampen auxin
transport (25, 29). It could, hence, be possible that an SL-in-
duced change in auxin transport is the reason for an effect of SL
signaling on cambium activity. However, several observations
support an influence of SLs on cambium activity, which is mainly
independent from regulating auxin transport. First, cambium activity is reduced in pin1 and pin3 mutants (Fig. 3), indicating
that, in general, basipetal auxin transport along the stem is
positively correlated with cambium activity. Second, even though
cambium activity is decreased, auxin concentration and signaling
is increased in the stem of max1-1 mutants. Third, genetic
analyses of the interaction between SL and auxin signaling in the
case of cambium regulation are in line with a requirement of
SL signaling for auxin becoming fully active on cambium cells
and show that SL responsiveness is not impaired in auxin signal-
ing mutants (Fig. 4). Moreover, the axr1-3 max1-1 and axr1-3 max2-1 double mutants did not display additive phenotypes in
comparison with single mutants arguing for a linear relationship
of both signaling pathways.
Recently, it was shown that auxin induces the expression of the SL
biosynthesis genes MAX3 and MAX4 in the stem of Arabidopsis in
an AXR1-dependent manner (51). In agreement with that observation, we propose that the reduced responsiveness of axr1-3 and
max1-1 toward NPA is, at least partly, due to a reduced SL bio-
synthesis. In the case of axr1-3, this model would imply that
exogenous SL applications can bypass auxin signaling in the in-
duction of secondary growth. In the case of max1-1, GR24
treatments would stimulate cambium activity largely independ-
dently from an effect on the auxin signaling pathway. Together
with the idea that there is SL production in the shoot (3) and the
observation that the expression of MAX2 in the cambium is suf-
ficient to restore wild-type–like levels of cambium activity (Fig. 4),
these results argue for an important role of auxin-dependent
stimulation of SL biosynthesis in the cambium for secondary
growth. As the evidence presented in this study is rather indirect,
we want to point out, however, that our interpretation does not
exclude the possibility that SLs influence cambium activity also
by regulating auxin transport.
Importantly, the reduction of cambium activity in the pea
mutant rms1-1 (impaired in SL biosynthesis) as well as the posi-
tive effect of GR24 treatments on the production of secondary
vascular tissue in Eucalyptus, demonstrate that the role of SL in
cambium activity is conserved among species and that it repre-
sents a common signaling mechanism for coordinating growth of
different plant organs.

Conclusions
The converse effects of SLs on branching and secondary growth
suggest a central role for SL in regulating plant architecture. Shoot branching and secondary growth are both influenced by
environmental stimuli such as shading, temperature, or day
length (52–55). On the basis of our finding that SLs are involved
in the regulation of both these processes, we hypothesize that
SLs function as modulators used by plants to switch between two
growth forms: a bushier form displaying a strong outgrowth of
many side shoots and a weak main stem, and a form in which the
main shoot dominates and displays enhanced secondary growth.
The ability to oscillate to a certain extent between these growth
forms could be regarded as being beneficial if, for example,
differences in the necessity to compete for light, or in the total
number of flowers produced by the shoot system under changing environmental conditions, are considered. This feature could represent a strategy in which the plant can choose the optimal way to grow to cope with the challenges imposed by a sessile lifestyle.

Materials and Methods

Details about growth conditions, cloning and plant lines, histological analyses, pharmacological treatments, RT-PCR, auxin measurements, and other techniques are in SI Materials and Methods. Arabidopsis thaliana (L.) Heynh, plants of the accession Columbia were used with the exception of the PXY:GUS reporter line, which is in the Ler background (38).

ACKNOWLEDGMENTS. We are grateful to Ottoline Leyser for providing the max1-1, max2-1, max3-9, max1-1, axr-1 max1-1, and axr-1 max2-1 mutant lines; Christian Luschning for providing the pin3-5 mutant; and Simon Turner for providing the PXY:GUS marker line. This work was supported by Austrian Science Fund (FWF) Grants P22394-B10 to M.L.S., P.S., and J.A.D.; the Wiener Wissenschafts-, Forschungs-, und Technologiefonds (T.S.), the Australian Research Council (E.A.D., P.B.B., and C.A.B.), and the Swedish Governmental Agency for Innovation Systems and the Swedish Research Council (K.L.).
Supporting Information

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SI Materials and Methods

Plant Material. Plants were initially grown under standard growth conditions for 3 wk in short day conditions (8 h light, 16 h dark) and then shifted to long-day conditions (16 h light, 8 h dark) to induce flowering. For pea analyses, wild-type and mst1-11 (MST-988) Térèse Pisum sativum plants were grown as described (1). Eucalyptus globulus plants were grown in growth chambers under standard long-day conditions (16 h light, 8 h dark). Arabidopsis mutants not mentioned in the acknowledgments were ordered from the Nottingham Arabidopsis Stock Centre.

Cloning and Transgenic Lines. To generate the APL:MAX2 (pSH1) construct, the MAX2 ORF was amplified by PCR and cloned into a vector containing the APL promoter (2), using NcoI/PciI and PstI restriction sites. For the generation of NST3:MAX2 (pSH2), we amplified a 3,028-bp genomic fragment upstream of the NST3 start codon using the primers NST3for3 and NST3rev6 and a 523-bp genomic fragment downstream of the NST3 stop codon using the primers NST3for7 and NST3rev3. The fragments were cloned into the KpnI and NotI restriction sites of pGreen0229 (3). The MAX2 ORF was cloned into NcoI/PciI and BamHI restriction sites generated between both promoter fragments. To produce the SCR:MAX2 (pSH3) construct, we amplified a 2,943-bp genomic fragment upstream of the SCR start codon and a 554-bp genomic fragment downstream of the SCR stop codon were amplified using the primer pairs SCRprom3/SCR Prom R and SCR Prom3_1/F/SCR Prom3_R, respectively. The fragments were cloned into pGreen0229. The MAX2 ORF was cloned into BamHI and XmaI restriction sites generated between both promoter fragments. To generate the WOX4:MAX2 (pSH5) construct, we amplified a 2,943-bp genomic fragment upstream of the WOX4 start codon using the primers WOX4for1 and WOX4rev1 and a 646-bp genomic fragment downstream of the WOX4 stop codon using the primers WOX4for9 and WOX4rev2. The fragments were cloned into pGreen0229 and the MAX2 ORF was cloned into NcoI/PciI and BamHI restriction sites generated between both promoter fragments. To produce the APL:CFP (APL:ER-ECPF-HDEL, pPS10) construct, the ER-ECPF-HDEL fragment was cloned into the PstI and NcoI restriction sites of a vector containing the APL promoter (2). Including the targeting sequence for the endoplasmic reticulum (ER + the HDEL motif) reduces passive diffusion of the fluorescent protein. To generate the PXY:CFP (PXY:ER-ECPF-HDEL, pPS19) construct, a 3,012-bp genomic fragment upstream of the start codon of the PXY promoter was amplified using the primers PXYfor1 and PXYrev1 and a genomic fragment downstream of the PXY stop codon using the primers PXYfor2 and PXYrev2. Both PCR products were cloned into pGreen0229. Next, the ER-ECPF-HDEL fragment was cloned into the NcoI and XmaI sites generated between both promoter fragments. All constructs were sequenced and after plant transformation, single copy lines were identified by Southern analyses and representative lines were used for further investigations. All primers mentioned in this section are listed in Table S1.

Histological Analyses. Arabidopsis stem fragments were analyzed as described previously (2). If not noted otherwise, plants were 20 cm tall when harvested. For Arabidopsis and Eucalyptus samples, 10-μM sections were produced using a microtome, deparaffinized, stained with 0.05% toluidine blue (Applichem), and fixed with Entellan (Merck). Wild-type and mst1-1 pea stem sections of 80 μm were produced from the main stem of 62-d-old plants at the base of internode 2 (immediately above node 2, counting the cotyledonary node as node 0) using a vibratome (Leica VT1200 S). Sections were stained for 30 s with 0.05% toluidine blue and rinsed with water. All samples were visualized using standard light microscopy. For qualitative analyses of both Arabidopsis and pea, at least five plants were analyzed for each data point (2, 4). The SE of means was used to visualize variation. Data were subjected to statistical analysis, using a two-tailed independent Student’s t-test with SPSS 18.0 software (http://www.spss.com). Significance levels of P < 0.05, P < 0.01, and P < 0.001 are indicated in the figures by single, double, and triple asterisks, respectively. GUS reporter activity was analyzed as described previously (5). GUS-stained samples were photographed using a Leica MZ 16 FA stereomicroscope and pictures were analyzed with Leica application software. For fluorescence analyses, free-hand cross-sections of stems were counterstained for 5 min in a 5 μg/mL propidium iodide (Merck) aqueous dilution and then mounted on microscope slides. For fluorescence analyses shown in Fig. 2, a LSM 710 Zeiss 2P spectral confocal microscope (Carl Zeiss) equipped with the Zen2009 version 5.5 SP1 software (Carl Zeiss) was used. Propidium iodide was excited at 514 nm and detected at 592–638 nm. CFP was excited at 458 nm and detected at 469–481 nm. Fluorescence analyses shown in Fig. 3 were performed using a Leica TCS-NT confocal microscope and the LP665 (barrier FW 4), RSP660 (detection beam splitter FW 3), and RSP500 (excitation beam splitter FW) filters (Leica Microsystems). Excitation of GFP and propidium iodide was achieved at 488 nm and detection at 500–520 nm and 610–630 nm, respectively.

Pharmacological Treatments. A stock solution of 10 mM GR24 (Chiraitx) dissolved in 100% acetone was kept at −20 °C. Aqueous solutions were obtained as previously described (6) and applied directly on stems with the help of a paintbrush. For Arabidopsis, 0-μM, 1-μM, or 5-μM treatments were performed in a region of 0.3 cm located 2 cm above the uppermost rosette leaf (within the first internode) of 7.5-cm-high plants, as described in Fig. 1C. The treatment was performed every 2 d and samples were collected after 21 d of incubation. For the N-1-naphthylphthalamic acid (NPA) treatments, a concentration of 10 mg/mL was applied in a ring using lanolin as a carrier. Samples incubated either with lanolin alone (mock) or with lanolin mixed with NPA were harvested after 14 d incubation and processed for histological analyses as described above. For Eucalyptus 0-μM, 5-μM or 10-μM treatments were performed in a region of 0.3 cm located at the central part of internode 2 (counting the cotyledonary node as node 0) on the main stem of 10-cm-tall plants. The treatment was performed every 2 d and samples were collected after 21 d of incubation.

RT-PCR. For RT-PCR, total RNA was extracted from the base of the stems of pSH1_1, pSH1_2, pSH2_1, pSH2_2, pSH3_1, pSH3_2, pSH5_1, and pSH5_2 plants and analyzed as described previously (4). Primers used for PCR were MAXfor7/APLrev24 for pSH1, MAXfor7/NST3rev7 for pSH2, MAXfor7/SCRrev6 for pSH3, and MAXfor7/WOX4rev15 for pSH5 lines. All primers are listed in Table S1.

qRT-PCR. Total RNA from the stem base of WT and max1-l mutants was extracted, purified, and analyzed as described (2, 4). cDNA production and qRT-PCR were performed as described (4). Cell cycle-related (ATGOLS1, AT1G76540, and AT5G62550) and
Cambium-specific genes (WOX4, AT5G57130, and MOL1) were selected on the basis of previous observations (4, 7, 8). For each sample, results obtained for a given gene were normalized using the value of an internal standard gene (EIF4; AT3G13920) on the basis of the comparative threshold (CT) method as described by Perkin-Elmer Applied Biosystems. Afterwards, the value for the given gene of interest in the control (wild-type) sample was set to 1.0 and the relative value for max1-1 was normalized accordingly. The specificity of the amplification reactions was assessed using postamplification dissociation curves. In all cases at least two biological and two technical replicates were carried out, resulting in four qRT-PCR reactions per gene and sample. Primers used were EIF4for/EIF4rev for EIF4 amplification, ATGOLS1for1/ATGOLS1rev1 for ATGOLS1 amplification, AT1G76540for1/AT1G76540 for AT1G76540 amplification, AT5G62550for1/AT5G62550rev1 for AT5G62550 amplification, WOX4for1/WOX4rev4 for WOX4 amplification, AT5G57130for2/AT5G57130rev2 for AT5G57130 amplification, and MOLfor2/MOLrev2 for MOL1 amplification.

PIN Gene Expression Data. Absolute expression for members of the PIN gene family in the second elongated internode was obtained from the AtGenExpress Visualization Tool (AVT) (9).

Auxin Measurements. Three-millimeter-long stem fragments were collected and immediately frozen in liquid nitrogen. The fragments were taken from the base of the stem immediately above the uppermost rosette leaf and from the central part of the internodes 1, 2, and 3. For each sample, material from 10 individual plants was combined. For each position and genotype, five biological replicates were analyzed. A total of 1 ng 13C6-indole-3-acetic acid (IAA) internal standard was added to each sample before extraction and purification as described previously (10). Free IAA was then quantified using gas chromatography-selected reaction monitoring-mass spectrometry (11).

Fig. S2. (A) Increasing amounts of GR24 lead to increased cell proliferation in interfascicular regions. (B) Local GR24 treatments have no effect on the outgrowth of side shoots in wild-type or max1-1 mutants. (C) Absolute expression for members of the PIN gene family in the second elongated internode according to the AtGenExpress Visualization Tool (AVT). (D) Analyses by RT-PCR show that transgenes are active in plant lines used for this study. RT-PCR using TUBULIN-specific primers (Bottom) demonstrated that all templates contained appropriate amounts of cDNA.
Fig. S3. The *pin1* and *pin3* mutants display reduced secondary growth at the base of the stem in comparison with wild type. (A–C) Cross-sections from immediately above the uppermost rosette leaf of wild type (*A*), *pin1-613* (*B*), and *pin3-5* (*C*). (D) Quantification of lateral interfascicular cambium-derived (ICD) extension immediately above the uppermost rosette leaf of wild type, *pin1-613*, and *pin3-5* as indicated in *A*–*C*. [Scale bar in *C* (100 μm) also applies to *A* and *B*.] Asterisks label the position of primary vascular bundles. Braces ([]) indicate the extension of the ICD.

Fig. S4. The *axr1-3* mutant displays reduced secondary growth in comparison with wild type. (A and B) Cross-sections from immediately above the uppermost rosette leaf of wild-type (*A*) and *axr1-3* (*B*). Asterisks indicate the position of primary vascular bundles. (C) Quantification of lateral ICD extension immediately above the uppermost rosette leaf of wild type and *axr1-3* as indicated in *A* and *B*. (D) Quantification of the longitudinal progression of IC initiation in 30-cm-tall plants starting from the uppermost rosette leaf.
Fig. S5. GR24 treatments stimulate cambium activity in *Eucalyptus globulus* stems. (A) Quantification of the production of vascular tissue shows a significant increase in plants treated with 10 μm GR24 in comparison with mock-treated plants. (B and C) Sections from *Eucalyptus* mock- (B) and GR24- (C) treated stems. [Scale bar in B (200 μm) also applies to C.] Braces ([]) indicate the lateral extension of the vascular tissues. (D) Quantification of the lateral cambium extension shows a significant increase in plants treated with 10 μm GR24 in comparison with mock-treated plants. (E and F) Stem sections from mock- (D) and GR24- (E) treated plants. Extension of the cambium (C) tissue is indicated by braces. [Scale bar in B (100 μm) also applies to C and scale bar in E (100 μm) also applies to F.]

Fig. S6. Analysis of cambium activity in pea. (A) Quantification of the ICD extension in wild type and *rms1-1* shows a significant reduction of cambium activity in *rms1-1*. (B and C) Sections visualizing the extension of the ICD in wild type (B) and *rms1-1* (C). [Scale bar in C (100 μm) also applies to B.]
Table S1. Primers used in this study

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