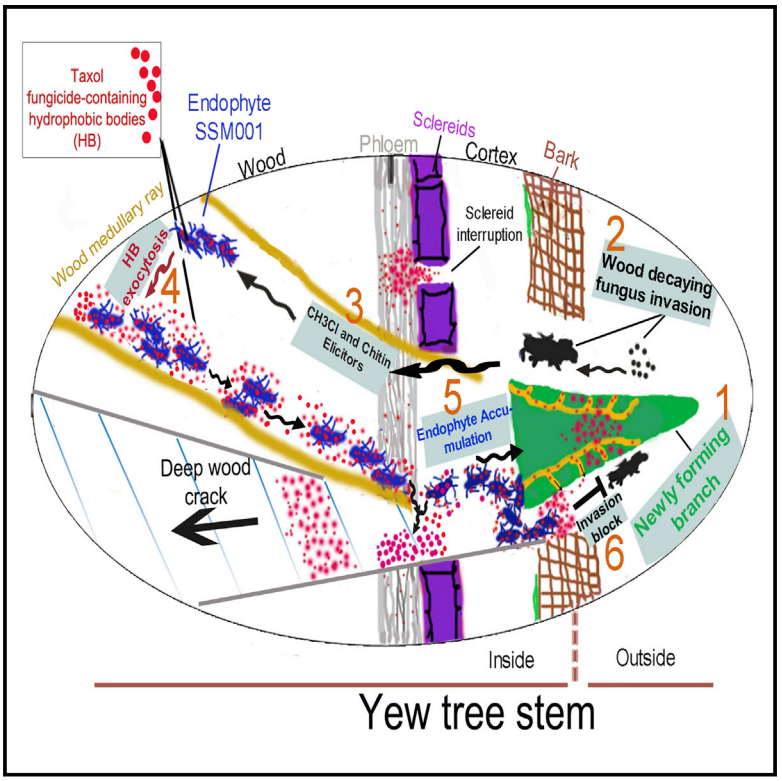
Report

Current Biology

An Endophyte Constructs Fungicide-Containing Extracellular Barriers for Its Host Plant

Graphical Abstract



Authors

Sameh S.M. Soliman,
John S. Greenwood,
Aureliano Bombarely, ..., Rong Tsao,
Dick D. Mosser, Manish N. Raizada

Correspondence

raizada@uoguelph.ca

In Brief

It has been a mystery why yew trees, which produce the cancer drug Taxol, host fungi that also produce Taxol, which in nature is a fungicide. Soliman et al. show that the fungi store Taxol in hydrophobic bodies, migrate to pathogen entry points, and then release the bodies upon sensing pathogens, to create fungicide-laced barriers for their host.

Highlights

- The cancer drug Taxol is a fungicide against wood-decaying fungi (WDF) in yew trees
- Inside yew, a fungal endophyte produces Taxol and migrates to pathogen entry points
- The endophyte sequesters Taxol in hydrophobic bodies (HBs); WDF induce their release
- The HBs can coalesce to form extracellular barriers laced with fungicidal Taxol

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An Endophyte Constructs Fungicide-Containing Extracellular Barriers for Its Host Plant

Sameh S.M. Soliman,[1,2] John S. Greenwood,[3] Aureliano Bombarely,[4] Lukas A. Mueller,[5] Rong Tsao,[6] Dick D. Mosser,[3] and Manish N. Raizada[1,*]

[1]Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1, Canada
[2]David Geffen School of Medicine at UCLA, Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, Torrance, CA 90502, USA
[3]Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON N1G 2W1, Canada
[4]Department of Horticulture, Virginia Tech, Blacksburg, VA 24071, USA
[5]SOL Genomic Network, Boyce Thompson Institute for Plant Research, Tower Road, Ithaca, NY 14853-1801, USA
[6]The Guelph Food Research Centre, Agriculture and Agri-Food Canada, Guelph, ON N1G 5C9, Canada
*Correspondence: raizada@uoguelph.ca
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SUMMARY

Surface cracks create sites for pathogen invasion. Yew trees (*Taxus*) hyperbranch from long-lived buds that lie underneath the bark [1], resulting in persistent bark cracking and deep air pockets, potentially allowing pathogens to enter the nutrient-rich vascular system (vertical phloem and inter-connected radial medullary rays [MR]). Yew is famous as the source of the anti-cancer diterpenoid drug Taxol. A mystery has been why both the tree and its resident non-pathogenic fungi (endophytes) synthesize Taxol, apparently redundantly [2–7]. These endophytes, as well as pure Taxol, suppress fungal pathogens including wood-decaying fungi (WDF) [8–11]. Here we show that a Taxol-producing fungal endophyte, *Paraconiothyrium* SSM001 [12], migrates to pathogen entry points including branch cracks. The fungus sequesters Taxol in intracellular hydrophobic bodies that are induced by WDF for release by exocytosis, after which the bodies can coalesce to form remarkable extracellular barriers, laced with the fungicide. We propose that microbial construction of fungicide-releasing hydrophobic barriers might be a novel plant defense mechanism. We further propose that the endophyte might be evolutionarily analogous to animal immune cells, in that it might expand plant immunity by acting as an autonomous, anti-pathogen sentinel that monitors the vascular system.

RESULTS

Branching in *Taxus* Causes Deep Wood Cracks in which a Taxol-Producing Endophytic Fungus Accumulates from Its Vascular Habitat

Surprisingly, at *Taxus* bark cracks, fungal hyphae hyperaccumulated in air pockets around developing buds (Figure 1D). When cracking was reproduced artificially by cutting the wood, the hyphae repeated this behavior and grew out from their native habitat, the medullary ray vascular system (Figures 1E and 1F). These hyphae were cultured (Figures S1A and S1B) and identified via 18S rDNA (Figure S1C) as an endophytic fungus that we previously classified as *Paraconiothyrium* strain SSM001 [12]. The fungus was tracked within *Taxus* by DNA fingerprinting (tRFLP) and observed to inhabit only wood (Figures S1E–S1J). This strain was previously shown to produce Taxol in vitro in the absence of its host [12].

Taxol and the Taxol-Producing Endophyte Inhibit Wood-Decaying Fungi In Vitro and in Intact Plants

Based on reports that Taxol is fungicidal in that it can suppress fungal mitosis [8–11], we tested whether endophyte SSM001 could suppress three wood-decaying fungi (WDF): *Heterobasidion annosum*, *Phaeolus schweinitzii*, and *Perenniporia subacida*. These WDF pathogens infect conifers, with *P. schweinitzii* known to infect *Taxus* [13–16]. SSM001 inhibited growth of these WDF in vitro (Figures 1G, S2A, and S2B) similar to their taxane extracts (Figure S2C) and pure Taxol (Figure 1H), but not solvent controls (Figure 1I). Pure Taxol did not inhibit SSM001 (Figure 1H) or regeneration of SSM001 mycelia from protoplasts (Figures S2D and S2E), demonstrating that SSM001 had evolved resistance that was not due to its cell wall acting as a barrier.

To determine whether SSM001 inhibits WDF in planta, *Taxus* plantlets were infected with *P. schweinitzii*, but only after a prior injection with a commercial fungicide to kill SSM001 (Figure 1J). PCR and tRFLP were used to monitor SSM001 (370 bp peak) and WDF (240 bp peak) (Figures S2F–S2T). The commercial fungicide caused a ~5-fold decrease in SSM001 compared to buffer-injected plantlets (Figure 1K). Pre-treated commercial fungicide plants showed significant increases in WDF compared to controls (Figure 1K). Combined with the in vitro and localization data, these observations suggest that SSM001 suppresses WDF in planta (e.g., at branch points).

Taxol Localizes to Hydrophobic Bodies within Endophyte SSM001

Electron microscopy revealed unusual hydrophobic bodies (HBs) within cultured hyphae of SSM001 (Figure 2A), an observation confirmed using Sudan IV, an orange lipophilic stain

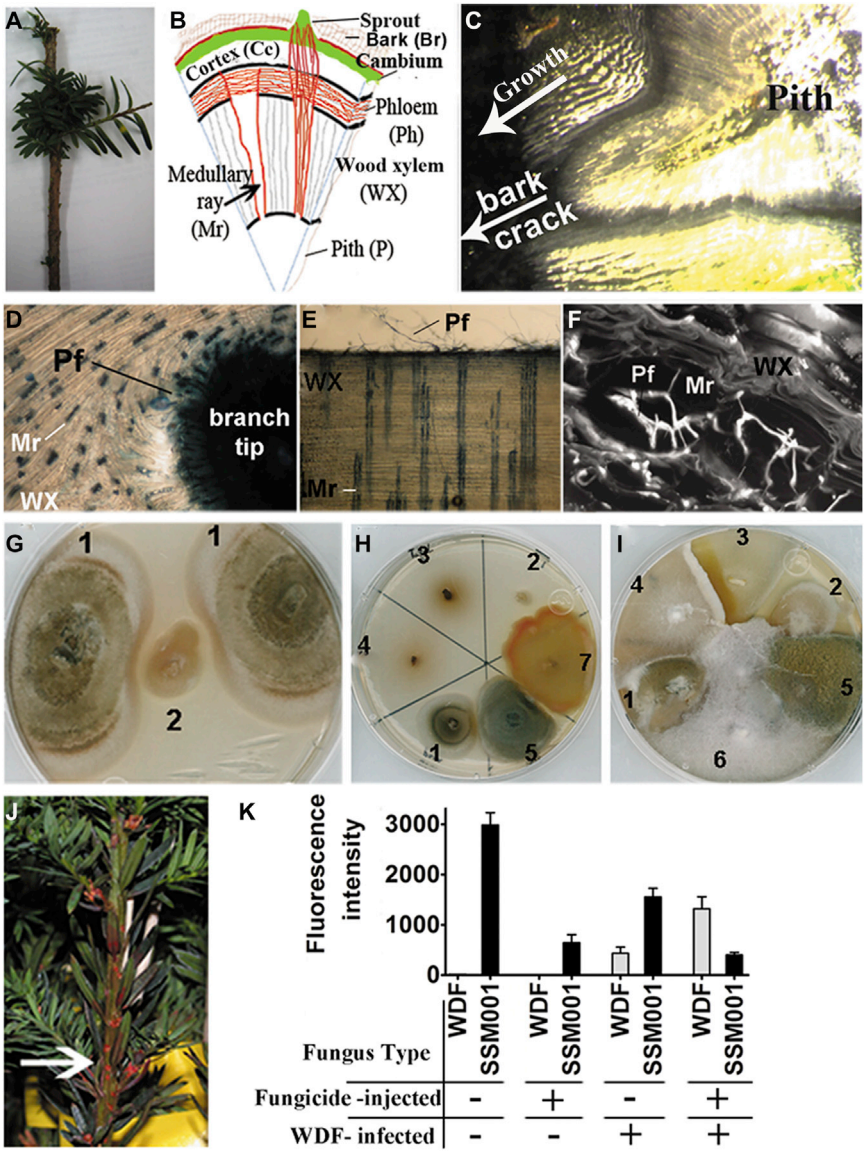

Figure 1. Persistent Branching of *Taxus* Plants and Accumulation of the Taxol-Producing Fungus *Paraconiothyrium* SSM001 at Wood Medullary Rays and *Taxus* Branch Points

(A) Photographs of persistent *Taxus* plant branching showing newly formed branch sprouting from stem cell populations underlying dead bark.

(B) Diagram of a transverse section of a *Taxus* branch describing the outer and inner layers.

(C) Light microscopy of a longitudinal newly formed branch section showing how the outer bark cracks open.

(D) Light microscopy of a longitudinal branch section showing the accumulation of *Paraconiothyrium* SSM001 (stained blue by Trypan blue) surrounding the emerging branch. The branch section was cultured on PDA for 12 hr, fixed, and stained with Trypan blue.

(E) Light microscopy of longitudinal wood sections cultured on PDA media for 72 hr, showing the localization and emergence of fungal hyphae in the wood medullary rays (WMR).

(F) Confocal microscopy of longitudinal wood sections cultured on PDA media for 18 hr showing localization of *Paraconiothyrium* SSM001 hyphae within wood medullary rays. See Figure S1 for details of SSM001 taxonomy, phylogenetic relationships, and in planta localization inside *Taxus* using tRFLP.

(G–K) Taxol and Taxol-producing fungus SSM001 are fungicidal against wood-decaying fungi (WDF). Shown are the fungicidal activities (G–I) in vitro and (J, K) in planta.

(G) Effect of SSM001 on WDF. *Paraconiothyrium* (#1) co-cultured alongside (#2) *Phaeolus schweinitzii* WDF showing WDF growth inhibition.

(H and I) Effect of pure Taxol standard (H) on WDF and other fungi in comparison to (I) methanol solvent control. Taxol-producing fungi (#1,6), non-Taxol-producing fungal endophytes (#5,7), and three WDF (#2,3,4). Taxol-producing fungi (*Paraconiothyrium* [#1] and *Pestalotipsis* [#6]), non-Taxol-producing fungi (*Alternaria* [#5], *Fusarium* [#7]), and WDF [#2,3,4]. Numbering is as follows: 1, *Paraconiothyrium* SSM001; 2, *Phaeolus schweinitzii*; 3, *Heterobasidion annosum*; 4, *Perenniporia subacida*; 5, *Alternaria*; 6, *Pestalotiopsis*; 7, *Fusarium graminearum*. For supporting data, see Figures S2A–S2E.

(J) Three-year-old *Taxus* plantlets were injected with fungicide (arrow and red color show the injection site).

(K) Graph showing quantitative comparisons of WDF (*Phaeolus schweinitzii*) and *Paraconiothyrium* SSM001 endophyte between fungicide-injected and non-fungicide-injected plantlets. Error bars represent SEM.

Abbreviations are as follows: Pf, *Paraconiothyrium* SSM001 hyphae; WX, wood xylem; Mr, medullary ray cells. WDF, wood decaying fungus. For the original data concerning the fungicide injections, see Figures S2F–S2T. For the results placed within an ecological model, see Figure S4.

(Figure 2B). Because both HBs and Taxol are hydrophobic, we hypothesized that HBs accumulate Taxol, which was confirmed by two experiments. First, immunogold labeling using a Taxol monoclonal antibody [17] demonstrated that Taxol accumulated inside HBs within SSM001 hyphae (13/18 images, Figure 2C) compared to controls. Second, in a time-course following SSM001 inoculation into liquid media, Taxol unexpectedly accumulated in the media which coincided with release of the HBs from the hyphae (at 3 weeks, Figures 2D–2H).

Taxol-Containing Hydrophobic Bodies Accumulate Extracellularly at Pathogen Entry Points

Staining with Sudan IV showed that HBs were present in the *Taxus* vascular system (Figure 2I) including the interconnected lateral medullary rays (Figure 2J) and vertical phloem (Figure 2K). Immunogold labeling using the Taxol monoclonal antibody confirmed that Taxol localized to the HBs within medullary rays (18/18 images, Figures S3A–S3C) compared to controls (Figure S3D).

Taxol-containing HBs were also observed where medullary rays traversed the sclereids (Figure 2L), a layer of cells that

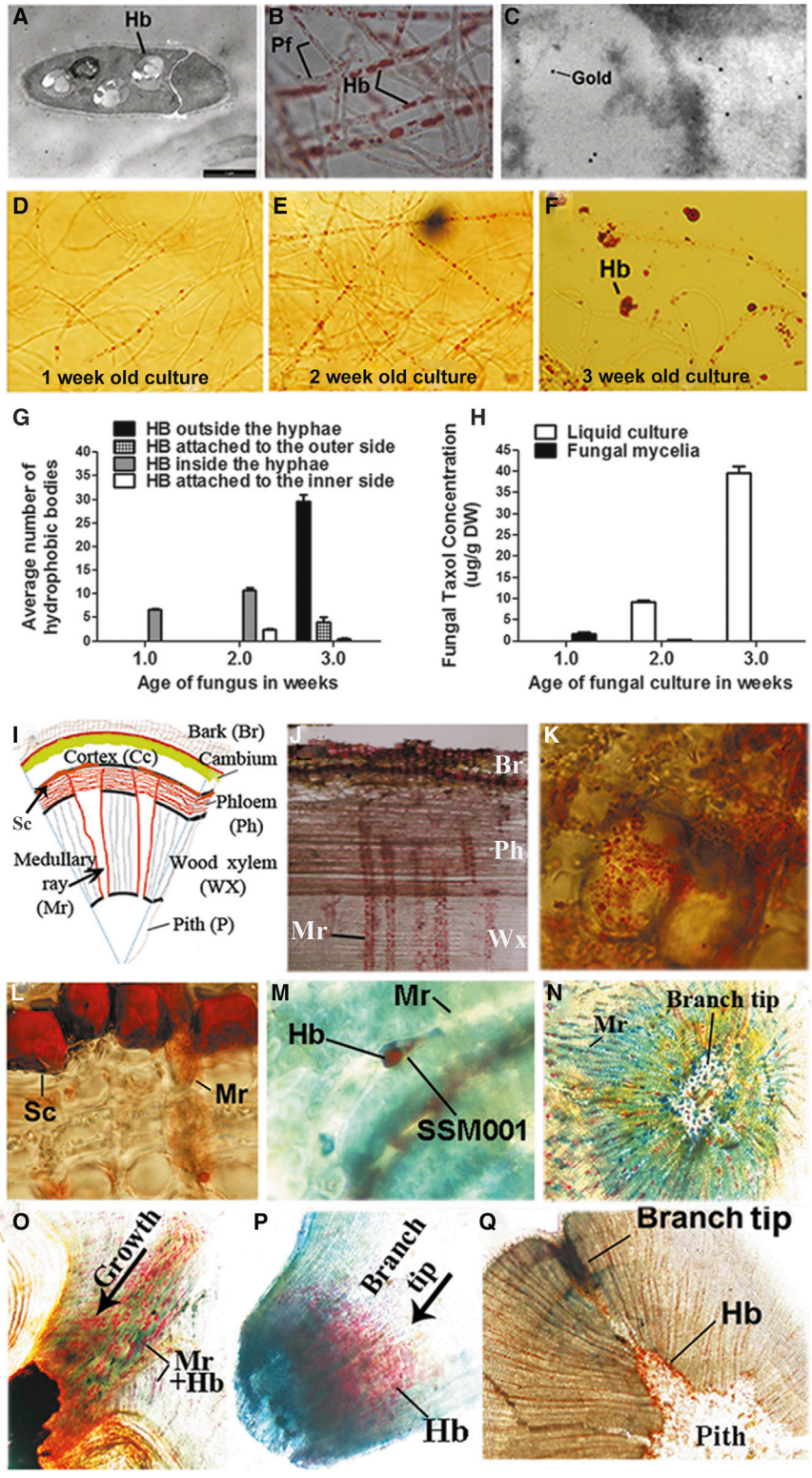

Figure 2. Taxol Accumulates within Hydrophobic Bodies in *Paraconiothyrium* SSM001

(A) Electron microscopy of SSM001 hyphae showing hydrophobic bodies.

(B) Light microscopy of hydrophobic bodies in *Paraconiothyrium* hyphae. The hydrophobic bodies stained orange with Sudan IV. For evidence that the hydrophobic bodies sequester the cytotoxic effects of Taxol from *Taxus* cells (as well as yeast and human cells), see Figures S2U–S2X.

(C) Transmission electron micrograph (TEM) of fungal hyphae treated with an anti-Taxol antibody showing immunogold localization of fungal Taxol within hydrophobic bodies. For immunogold localization of Taxol in hydrophobic bodies within wood medullary rays, see Figures S3A–S3D.

(D–F) Time course accumulation of hydrophobic bodies inside and outside SSM001 fungal hyphae at (D) 1 week, (E) 2 weeks, and (F) 3 weeks of age.

(G and H) Time course accumulation of (G) hydrophobic bodies and (H) fungal Taxol, at different fungal ages after inoculation in liquid culture, showing coincident accumulation of both hydrophobic bodies and Taxol in the culture media. Error bars represent SEM.

(I–Q) *Paraconiothyrium* SSM001 and Taxol-containing bodies accumulate primarily at potential pathogen entry points of *Taxus*.

(I) Diagram of a transverse section of a *Taxus* branch stem.

(J) Light microscope image of a transverse section of a *Taxus* branch showing the accumulation of hydrophobic bodies within the phloem and wood medullary rays. The hydrophobic bodies stained orange to red with Sudan IV, and their edges stained blue with Trypan blue.

(K) Closeup view of the hydrophobic bodies in the phloem.

(L) The sclereid layer (horizontal, purple) interrupted by phloem medullary ray cells (vertical) containing Taxol-containing bodies (orange, Sudan IV stain).

(M–Q) Light microscope images of *Taxus* plant sections stained with a chitin-specific stain (blue, Lactophenol Cotton blue) and a hydrophobic body-specific stain (orange-red, Sudan IV).

(M) Closeup view of longitudinal section showing strong blue staining in the WMR owing to the SSM001 hyphae and HBs localized to it.

(N–P) Top and side views of newly formed *Taxus* branches showing the coincident accumulation of both SSM001 hyphae (stained blue with Lactophenol blue) and hydrophobic bodies (stained red with Sudan IV).

(Q) Light microscope image of a freshly stained longitudinal *Taxus* section at a newly formed branch showing accumulation of hydrophobic bodies (orange Sudan IV stain) at the branch point with a nearby zone of apparent plant cell death (Evan's blue stain).

Abbreviations are as follows: Hb, hydrophobic bodies; Ph, phloem cells; Br, outer bark; Cc, cortex; WX, wood xylem cells; Mr, medullary ray cells; Pf, *Paraconiothyrium* SSM001 hyphae; Sc, sclereids. For evidence that the hydrophobic bodies sequester the cytotoxic effects of Taxol from *Taxus*, yeast, and human cells, see Figures S2U–S2X. For the results placed within an ecological framework, see Figure S4.

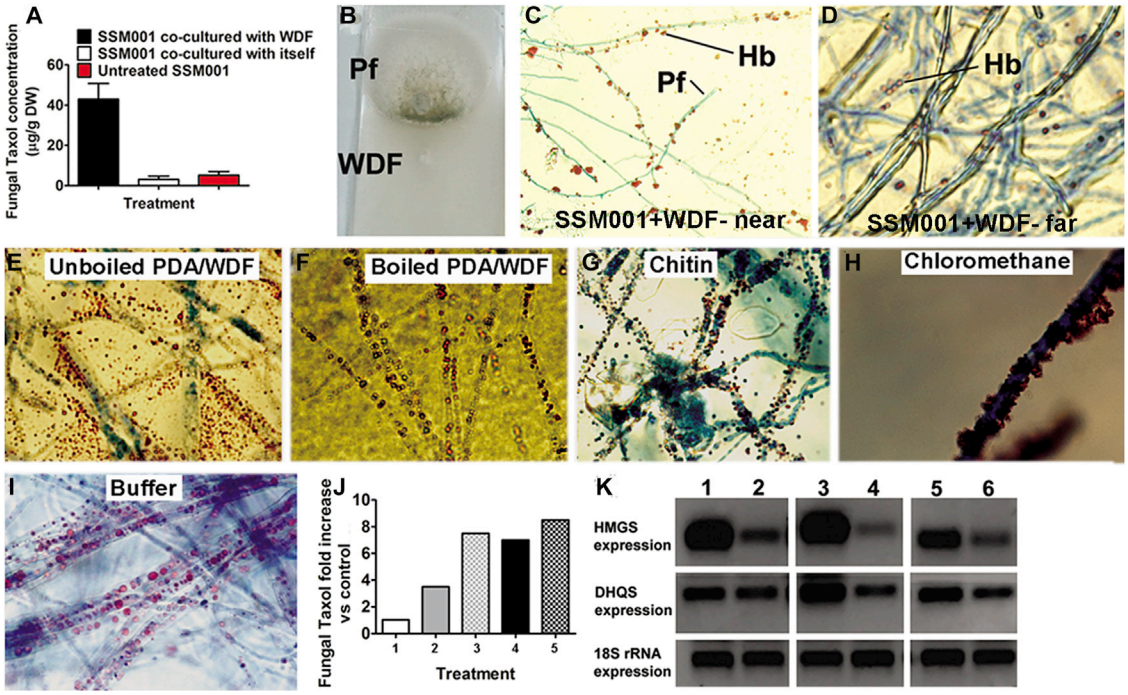

Figure 3. Wood-Decaying Fungus and Its Diffusible Chemicals, Chloromethane and Chitin, Induce Taxol Biosynthetic Gene Expression and the Release of Taxol-Containing Hydrophobic Bodies from *Paraconiothyrium* SSM001

(A) Quantification of fungal Taxol in liquid cultures of SSM001 co-cultured with either WDF or itself. Error bars represent SEM.

(B) Co-culture of WDF and SSM001 on a microscopic slide coated with a thin-film of PDA media showing green accumulation within SSM001 interfacing WDF.

(C and D) Closeup views of (C) SSM001 hyphae interfacing with WDF and (D) SSM001 hyphae located away from the WDF.

(E–I) Effects of various treatments on the release of Taxol-containing bodies from *Paraconiothyrium* SSM001 as stained with Trypan blue and Sudan IV.

(E and F) Shown are the effects of methanol:water (1:2) extracts of (E) unboiled PDA previously cultured with a WDF (*Phaeolus schweinitzii*) and (F) boiled PDA previously cultured with *P. schweinitzii*.

(G–I) Effects of adding known WDF-diffusible chemicals on the release of Taxol-containing bodies from *Paraconiothyrium* SSM001. Shown are the effects of adding (G) chitin fragments suspended in DMSO:methanol (1:1), (H) chloromethane, or (I) buffer (DMSO:methanol) as a control.

(J) Quantification of fungal Taxol in liquid cultures of *Paraconiothyrium* SSM001 treated with either chloromethane (8 or 16 mM), chitin (166 or 332 mg/l), or solvent methanol. In lane 1, SSM001-treated solvent control; in lane 2, SSM001-treated chitin at 166 mg/l; in lane 3, SSM001-treated chitin at 332 mg/l; in lane 4, SSM001-treated CH_3Cl at 8 mM; and in lane 5, SSM001-treated CH_3Cl at 16 mM. For additional details and controls, see Figures S3E–S3H.

(K) Fungal Taxol biosynthetic gene expression (RT-PCR) in response to pathogenic WDF, chloromethane, and chitin, specifically the expression of fungal 3-hydroxymethyl glutyryl CoA synthase (HMGS) in the mevalonate pathway, and fungal 3-dehydroquinate synthase (DHQS) in the shikimate pathway. We have previously implicated both the cytosolic mevalonate (MVA) pathway and the shikimate pathway as being involved in fungal Taxol biosynthesis based on quantitative chemical inhibitor studies [12]. The fungal PCR primers were based on 454 EST transcriptome sequencing of *Paraconiothyrium* SSM001 (see Supplemental Experimental Procedures). See Figures S3I and S3J for the DNA sequences. In lane 1, the WDF is *Phaeolus schweinitzii*; in lane 3, 16 mM chloromethane was used; and in lane 5, 332 mg/l chitin was used. Lanes 2, 4, and 6 are buffer control.

For the results placed within an ecological framework, see Figure S4.

protects phloem from breaks in outer tissues. Co-staining with chitin stain Lactophenol Cotton Blue showed that HBs within the rays localized to the endophyte (Figure 2M).

At branch points, branch primordia hyper-accumulated the endophyte and its HBs (Figure 2N), but as primordia developed, the endophyte released the HBs, accumulating extracellularly near branch tips, even coalescing (Figure 2O). Because branch primordia are fed by the vascular system (Figure 2P), they are nutrient-rich pathogen targets. When deep cracks formed associated with branch emergence, the HBs similarly released from vascular cells and coalesced extracellularly, forming barriers that sealed the vascular system (Figure 2Q).

Wood-Decaying Fungus Induces Taxol Accumulation In Vitro and the Release of Taxol-Containing Hydrophobic Bodies

When SSM001 was pre-cultured on agar with the WDF *P. schweinitzii*, Taxol production increased after subsequent inoculation of SSM001 into liquid media (Figure 3A); when SSM001 was pre-co-cultured with itself, Taxol was not significantly greater than untreated SSM001 controls (Figure 3A). To investigate this further, SSM001 was grown next to WDF on microscope slides (Figure 3B); interestingly, Taxol-containing HBs were released from SSM001 hyphae at the pathogen interface (Figure 3C) but not on the opposite control side (Figure 3D), indicating that WDF induce release of Taxol-containing HBs.

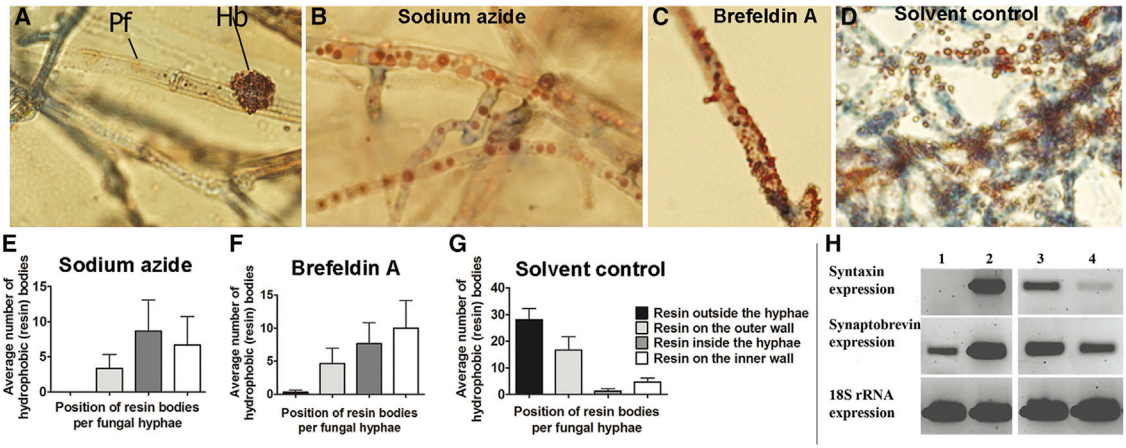

Figure 4. Fungal Taxol-Containing Bodies Are Released from *Paraconiothyrium* SSM001 by Exocytosis, not Passively via Fungal Cell Death

(A) Strain SSM001 stained with Sudan IV (orange, Taxol-containing bodies) and Trypan blue (fungal cytoplasm stain) at higher magnification, showing induced hydrophobic bodies release from hyphae. The image demonstrates that intact endophytic hyphae release hydrophobic bodies, suggesting that fungal cell death is not required for this release.

(B–D) Assay for hydrophobic body release from strain SSM001 co-cultured with WDF in the presence of the exocytosis inhibitors, (B) 10 mM sodium azide, or (C) 30 μg/ml Brefeldin A, compared to (D) solvent control. Both exocytosis inhibitors dramatically reduce hydrophobic body release compared to the buffer control.

(E–G) Quantification of the locations of hydrophobic bodies after treatment with either (E) sodium azide, (F) Brefeldin A, or (G) the buffer control. Error bars represent SEM.

(H) Effects of fungal culture age and a WDF candidate elicitor (chloromethane) on the expression of two fungal genes, Syntaxin and Synaptobrevin, which encode exocytosis machinery proteins (SNARE complex). Lane 1, untreated 1-week-old *Paraconiothyrium* SSM001; lane 2, untreated 3-week-old SSM001; lane 3, chloromethane-treated SSM001; lane 4, buffer control-treated SSM001. The fungal PCR primers for Syntaxin and Synaptobrevin were based on 454 EST transcriptome sequencing of strain SSM001 (see Supplemental Experimental Procedures).

See Figures S3K and S3L for the DNA sequences. For the results placed within an ecological framework, see Figure S4.

To test whether WDF produce diffusible elicitor(s), *P. schweinitzii* was cultured on PDA agar; water:methanol extracts of the agar, either boiled or unboiled, were then added to liquid cultures inoculated with SSM001. The unboiled fraction increased release of Taxol-containing HBs (Figure 3E). Unboiled extracts from agar exposed to WDF also increased fungal Taxol in liquid media fractions compared to boiled controls (Figure S3E). Taxol-containing HBs were not released by adding either boiled fractions (Figure 3F) or the boiled and unboiled agar previously exposed to SSM001 (Figures S3F and S3G) or unboiled plain agar (Figure S3H).

A candidate approach was undertaken to identify chemical elicitors from WDF [18] including chitin, known to stimulate Taxol [19]. A more specific candidate was chloromethane, a compound produced by WDF including *P. schweinitzii* during wood decay (lignin degradation) [18, 20]. Chloromethane was attractive, because dichloromethane is a well-known Taxol solvent [21] and its gaseous nature would permit diffusion through bark cracks—ideal for early pathogen detection. In fact, both chitin (Figure 3G) and chloromethane (Figure 3H) dramatically increased release of HBs compared to controls (Figure 3I). These data suggest that WDF and its metabolites induce release of Taxol-containing HBs from endophyte SSM001.

Elicitors from Wood-Decaying Fungus Upregulate Genes Required for Fungal Taxol Biosynthesis

Chitin and chloromethane added into endophyte SSM001 liquid media increased Taxol accumulation compared to buffer (Figure 3J). Mechanistically, pathogenic WDF, chitin, and chloromethane upregulated SSM001 genes critical to metabolic pathways required for endophytic Taxol production [12], specifically orthologs of the mevalonate pathway gene, HMGCoA synthase (HMGS), and the shikimate pathway gene, 3-dehydroquinate synthase (DHQS) (Figures 3K, S3I, and S3J).

Taxol-Containing Hydrophobic Bodies Are Released by Exocytosis

We hypothesized that Taxol-containing HBs are released by hyphal cell death (apoptosis) or exocytosis [22]. In the presence of pathogenic WDF, living hyphae, not dead cells, released the HBs, counter to the apoptosis hypothesis (Figure 4A). In the presence of pathogenic fungi and fungal exocytosis inhibitors [23], specifically sodium azide (Figures 4B and 4E) and Brefeldin A (Figures 4C and 4F), HBs remained primarily within the fungal hyphae compared to buffer controls (Figures 4D and 4G). Expression of SSM001 orthologs of Syntaxin and Synaptobrevin (Figures S3K and S3L), SNARE genes that encode the exocytosis machinery [24], were dramatically upregulated in 3-week-old SSM001 compared to 1-week-old cultures (Figure 4H); these time points coincided to high versus low HB and Taxol release, respectively (Figures 2G and 2H). Similarly, chloromethane caused upregulation of both SNARE orthologs compared to controls (Figure 4H). Combined, these results suggest that diffusible chemical signals from WDF induce exocytosis of fungal Taxol-containing HBs.

Hydrophobic Bodies Sequester and Protect *Taxus* Cells from Taxol

Endophyte SSM001 was immune to Taxol (Figures 1H, S2D, and S2E), yet pure Taxol injected into *Taxus* plantlets caused whole-plant toxicity compared to buffer injections (Figure S2U), consistent with reports that Taxol disrupts plant cell cytokinesis [25]. We tested whether HBs could facilitate sequestration of Taxol away from dividing plant cells such as in branch primordia (Figures 1B and 1C) where SSM001 accumulated (Figures 2N–2Q). A Taxol-sensitive yeast strain was exposed to pure Taxol or hydrophobic extracts (resin) embedded with Taxol; only pure Taxol, without the hydrophobic extract, was toxic (Figures S2V and S2W). Human acute lymphoblastic T cells (PEER) [26] were exposed to pure Taxol or Taxol-embedded hydrophobic extract; pure Taxol inhibited growth of PEER cells but the effect decreased when embedded into fungal hydrophobic extract (Figure S2X). These results suggest that HBs sequester endophytic Taxol to protect dividing plant cells.

DISCUSSION

Yew trees are likely to have inherent limitations with respect to protecting themselves against pathogen invasions at branch-associated bark cracks, including through the use of plant-derived Taxol as a natural fungicide. First, because Taxol inhibits cell division [25], its release within/near branch primordia would stop primordia growth. Second, local plant cell suicide to prevent pathogen spread (hypersensitive response) [27] would prevent continued plant production of Taxol or other anti-fungal compounds. Third, callose deposition to block pathogen entry into the vascular system [28] would starve growing branches. Finally, it is difficult to imagine how plants, including yew, can combat pathogens at air pockets (e.g., surrounding emerging branches).

This study suggests that yew formed symbiotic relationship(s) with Taxol-producing endophyte(s) [12] to solve the above problems through an intricate series of steps (summarized, Figure S4). In response to elicitors from wood-decaying fungi (WDF), including potentially crack-diffusing chloromethane, endophyte SSM001 upregulates fungicidal Taxol synthesis (Figure 3). The endophyte protects dividing plant cells, abundant in branch primordia, from Taxol phytotoxicity by sequestration in hydrophobic bodies (HBs) (Figures 2A–2F and S3A–S3D). These fungicide-laced HBs are then released by exocytosis in response to the pathogen elicitors (Figure 4). The HBs target pathogen entry points (deep bark cracks, sclereid interruptions, wood vascular system, nutrient-rich young buds) (Figures 1 and 2), where they can coalesce extracellularly to seal them (Figure 2Q). Microbial production of fungicide-containing hydrophobic barriers appears to be a novel host immunity mechanism. Thus, whereas plant Taxol presumably assists general immunity, endophytic Taxol might target immunity where plant cells cannot (Figure 2), perhaps finally explaining why both organisms produce Taxol. Because plant cells cannot move, endophytes such as SSM001 might have been recruited by plants to fulfill the mobile, autonomous, vascular-sentinel function (Figures 1D–1F and 2) provided by immunity cells in animals, in this case to combat WDF (Figures 1G–1K).

As for how Taxol is released from the HBs, as noted earlier, dichloromethane is a good solvent for Taxol [21], and because WDF produce this volatile compound (chloromethane), the pathogen itself might solubilize its own poison but only locally, minimizing exposure of plant cells to this cytokinesis inhibitor.

As to why yew selected an endophyte that uses Taxol as its fungicidal mechanism, both partners might have been primed—sharing metabolic precursors for Taxol [12] and possibly having compatible signaling pathways.

Taxol-producing endophytes are inhabitants of two other gymnosperms, ginkgo [29] and Wollemi pine [30–32]. Interestingly, SSM001 from yew co-clusters phylogenetically with these endophytes (Figure S1D), suggesting common evolutionary origins. Ginkgo and Wollemi also have branch-forming stem cells that persist under the bark [1, 33–35]. Like *Taxus*, ginkgo and Wollemi are among the most unique species on Earth—living fossils dating back >100 million years [36]; the discovery of only 100 Wollemi trees in Australia in 1994 was considered the botanical discovery of the 20th century [37]. These observations lead us to hypothesize that Taxol-producing endophytes might protect the branch points of other resilient gymnosperms.

ACCESSION NUMBERS

The GenBank accession numbers reported in this paper from *Paraconiothyrium* strain SSM001 (Figure S3) are as follows: 3-hydroxy-3-methylglutaryl-CoA synthase (JZ896663), 3-dehydroquinate synthase (JZ896662), syntaxin (JZ896664), synaptobrevin (JZ896665).

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.08.027.

AUTHOR CONTRIBUTIONS

The authors made the following contributions to this work: S.S.M.S. designed and undertook all experiments and interpreted the results; M.N.R. assisted in the design of experiments and data interpretation; R.T. helped with HPLC-MS; J.S.G. helped with the immuno-localization of Taxol; D.D.M. helped with the anticancer assay; A.B. and L.A.M. performed transcriptome analysis; and S.S.M.S. and M.N.R. wrote and revised the manuscript.

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