

Supplemental material for the manuscript

Comparative ‘omics’ of the *Fusarium fujikuroi* species complex highlights differences in genetic potential and metabolite synthesis

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Key words: *Fusarium fujikuroi* species complex; genome sequencing; secondary metabolism; *in planta* expression; metabolomics; evolution

Figures

85 *Fusarium* PKS
comprise
27 different groups

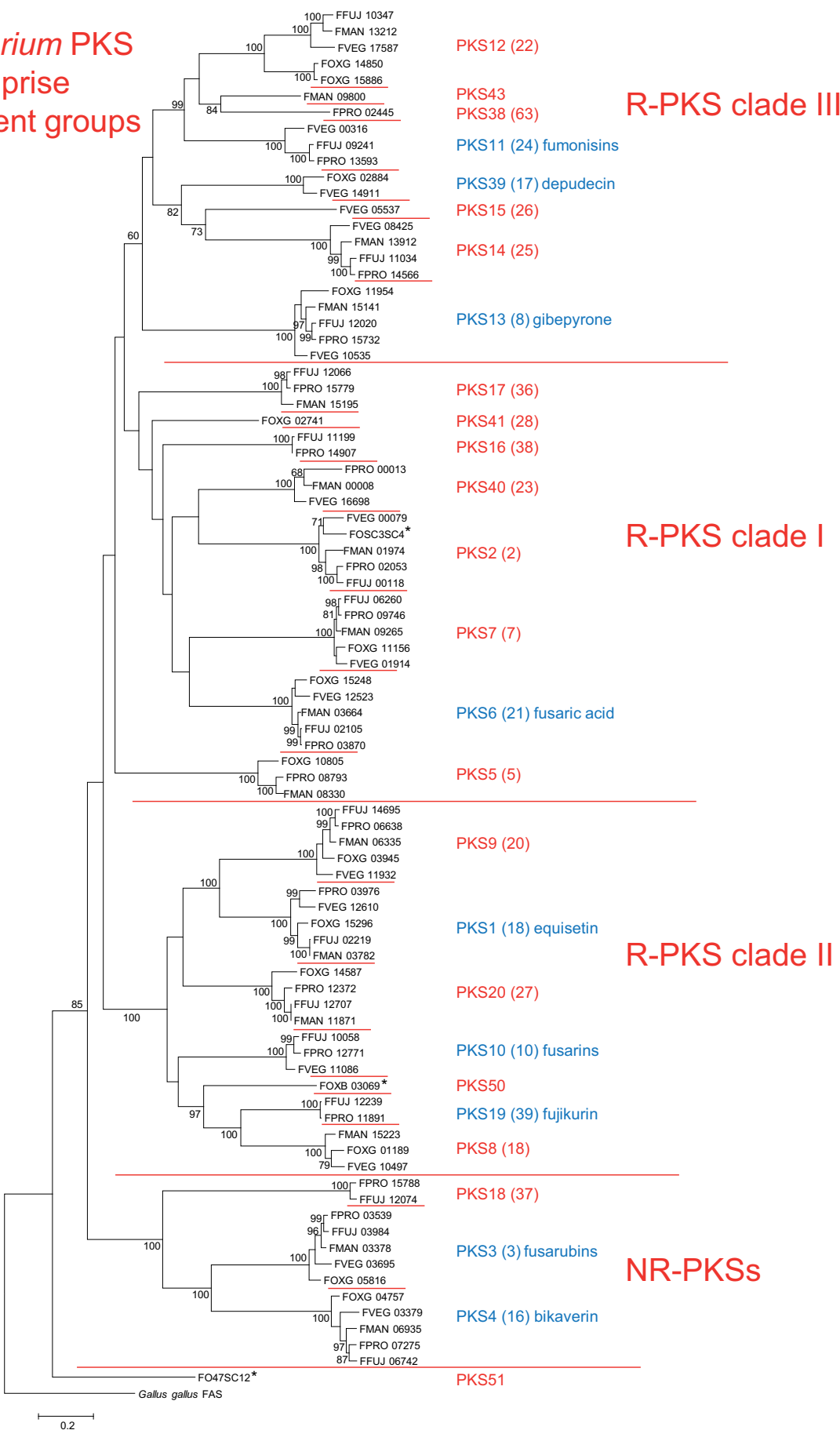


Fig. S1. Phylogram of 85 *Fusarium* PKS genes resolved into 27 homolog groups.

The phylogram was generated by maximum parsimony analysis of full-length coding region sequences of 85 PKS genes identified in the genomes of five members of the *Fusarium fujikuroi* species complex (FFC) and *F. oxysporum*. The NR-PKS and R-PKS I, II and III groups are delineated with long red horizontal lines, and the 27 homolog groups, or clades, are delineated with short red horizontal lines and are numbered with blue or red text to the right of the tree. The numbers outside parentheses correspond to PKS numbers described by Wiemann et al. (2013), while those within parentheses are homology group/clade numbers employed by Hansen et al. (2015) and Brown and Proctor (2016). The names of nine *Fusarium* metabolites are indicated in blue text adjacent to group/clade numbers. Numbers in black text near branch nodes are bootstrap values. The *Gallus gallus* fatty acid synthase gene served as outgroup.

* Three additional unique PKSs were present in three other *F. oxysporum* strains not examined in this study. *F. oxysporum* strain FOSC 3 contains the PKS FOSC3SC4, strain 5187 contains the PKS/NRPS FOXB_03069, and strain FO47 contains the PKS FO47SC12.

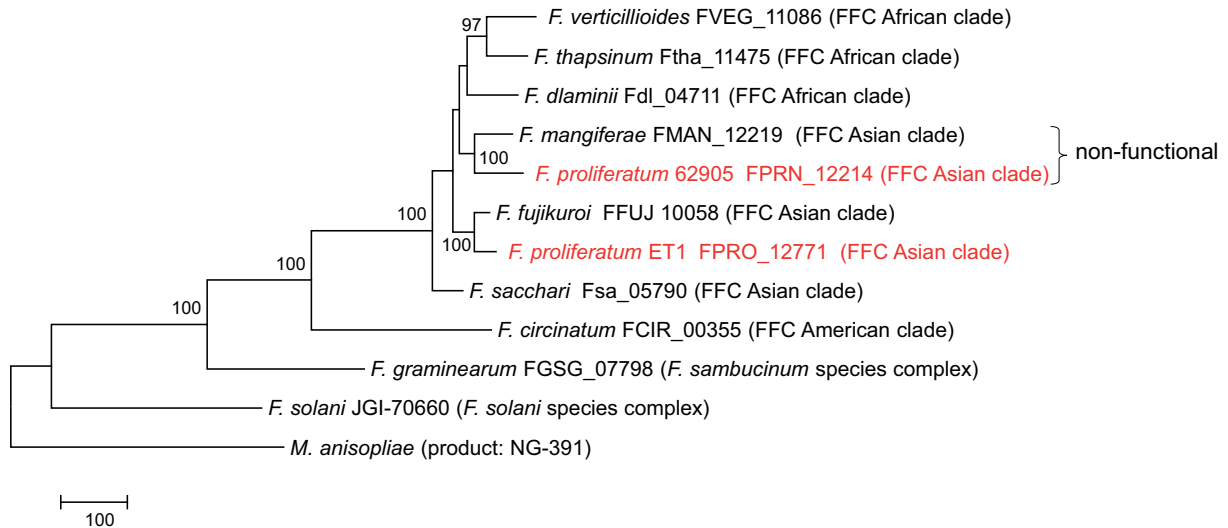


Fig. S2. Phylogram of functional and pseudogenized homologs of the fusarin PKS gene *FUS1* from 11 fusaria.

The phylogram was generated by maximum parsimony analysis of the region corresponding to nucleotides 6231 – 11751 of the *F. fujikuroi* *FUS1* coding region. The *FUS1* homologs in *F. mangiferae* (FMAN_12219) and *F. proliferatum* 62905 (FPRN_12214) have undergone a deletion of approximately half of the coding sequence and, therefore, are considered nonfunctional/pseudogenes (marked in red). Numbers in black text near nodes are bootstrap values. The *Metarhizium anisopliae* *FUS1* homolog served as outgroup.

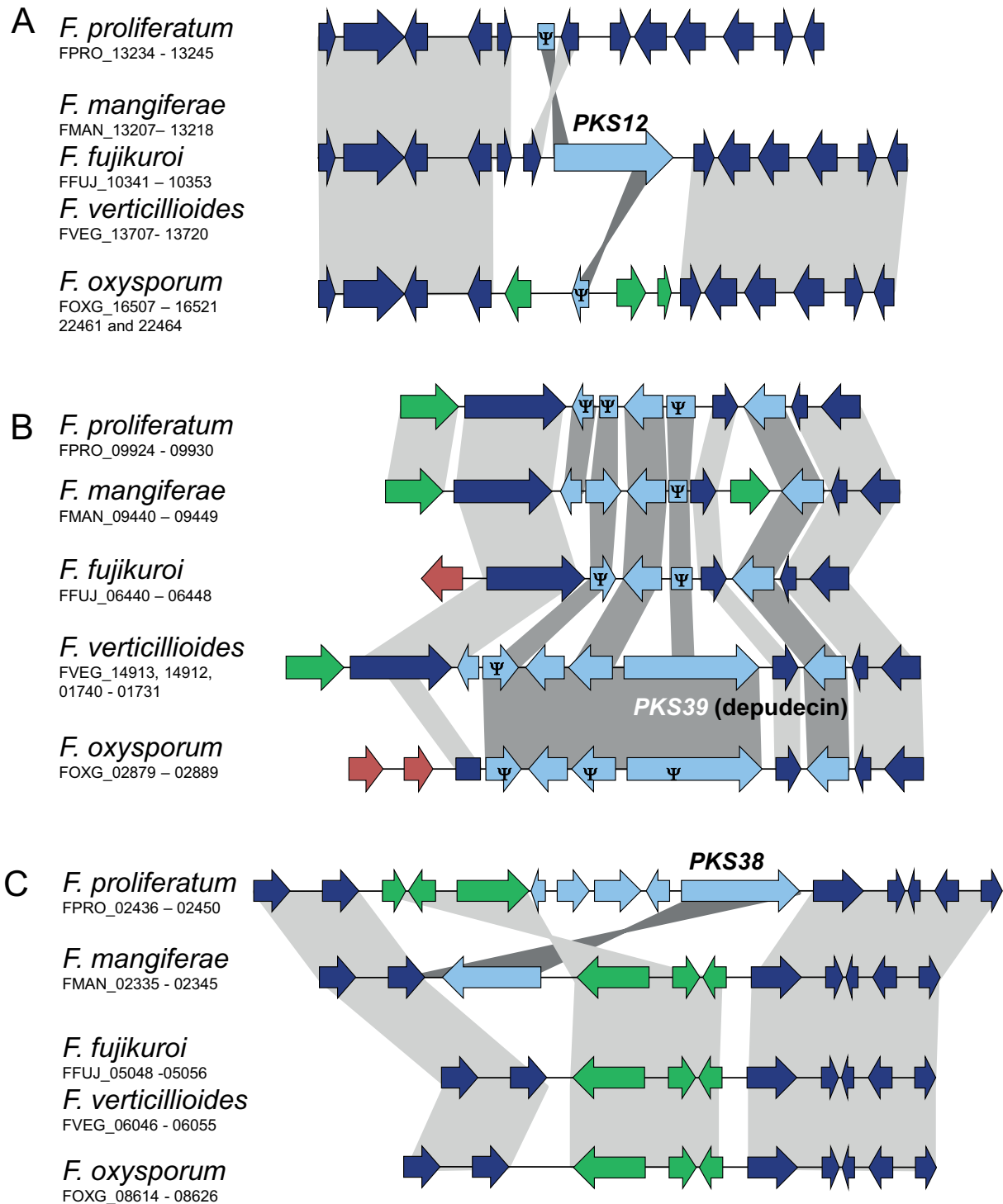
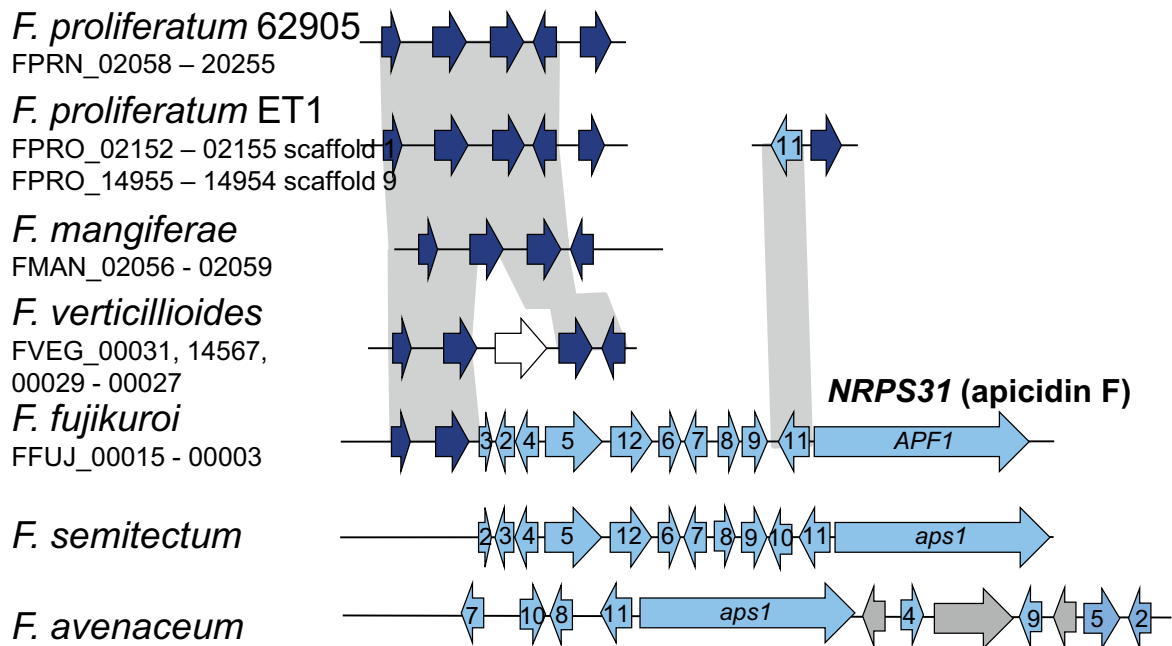


Fig. S3. Examples of likely recent acquisition of PKS genes.

(A) The PKS12 gene is intact in *F. fujikuroi*, *F. mangiferae* and *F. verticillioides*; but only a remnant of it is present in *F. oxysporum* and *F. proliferatum*. (B) The PKS39 cluster (light blue arrows), which is responsible for depudecin biosynthesis, exhibits various levels of degeneration among FFC members and *F. oxysporum*. For example, all but one of the cluster genes are intact in *F. verticillioides*, whereas only two genes are intact in *F. fujikuroi*. (C) The putative five-gene PKS38 cluster (light blue arrows) is unique to *F. proliferatum* among the species examined. However, the PKS38 gene is also present in *F. mangiferae*. Ψ indicates a pseudogene.

A



B

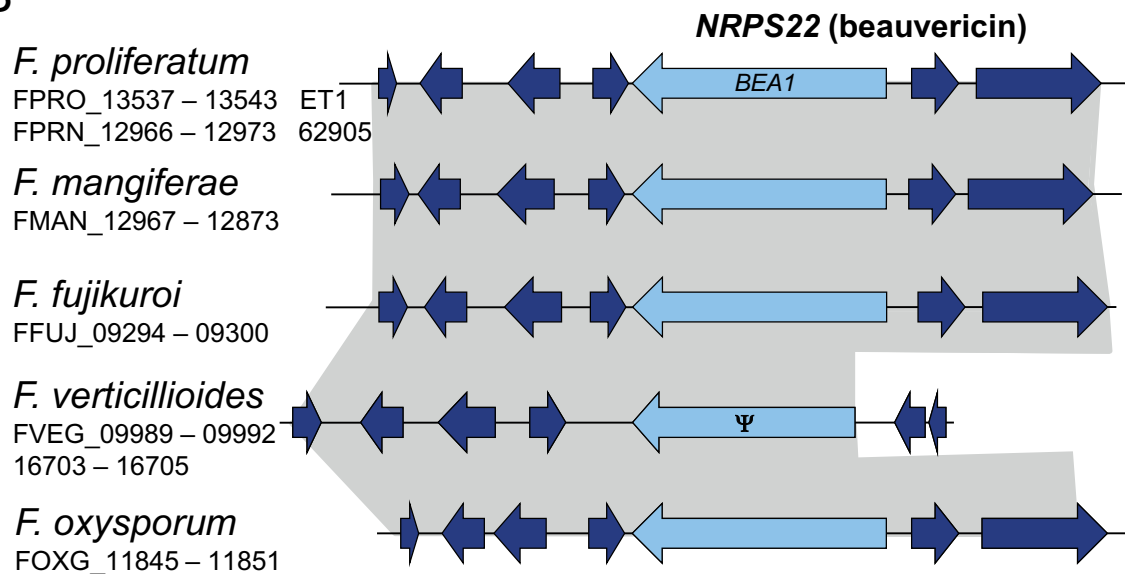
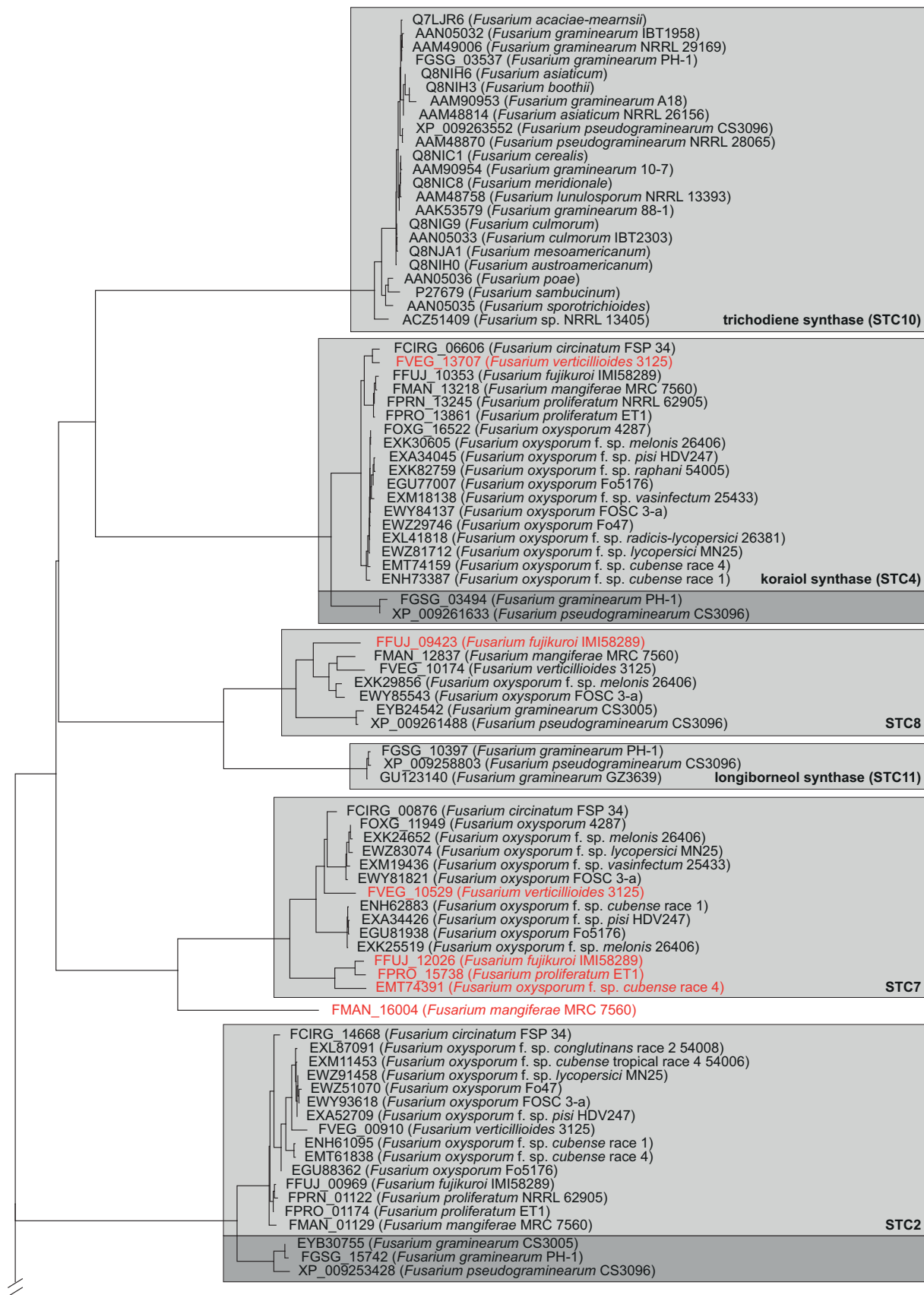


Fig. S4. Examples of the distribution of NRPS gene clusters in species of the FFC and *F. oxysporum*.

(A) The NRPS31 cluster (light blue arrows) is required for apicidin F (APF) synthesis in *F. fujikuroi* is an example of a likely recent acquisition of a NRPS cluster. The APF gene cluster is absent from the same genomic context in *F. proliferatum*, *F. mangiferae* and *F. verticillioides*. One of the cluster genes (*APF11*) is present at a different genomic context in *F. proliferatum* 62905; (B) The NRPS22 cluster (light blue arrows) involved in beauvericin synthesis (BEA) is present in the same genomic context in *F. proliferatum*, *F. mangiferae*, *F. fujikuroi* and *F. oxysporum* and similar context in *F. verticillioides*. Ψ indicates a pseudogene.



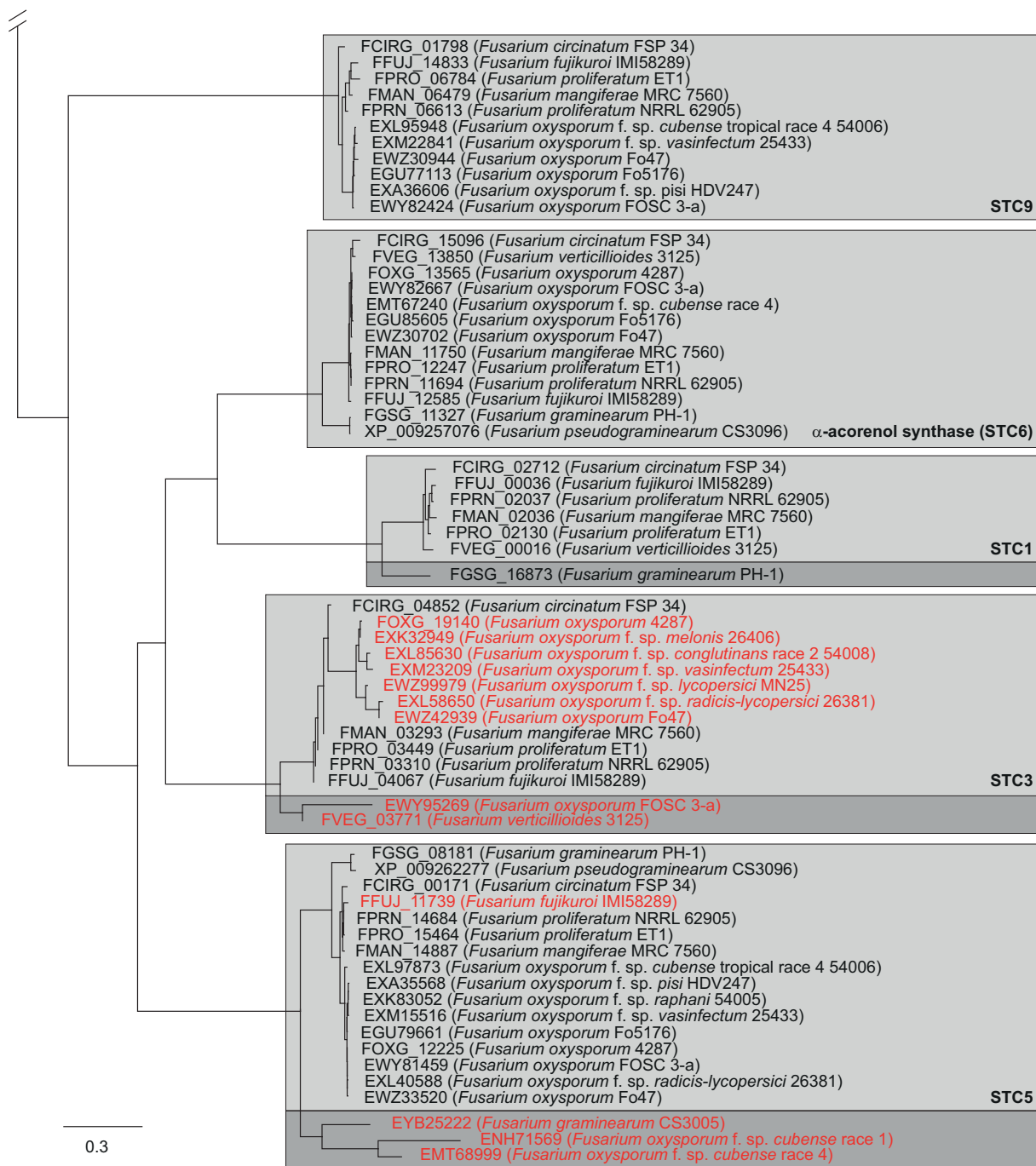


Fig. S5. Phylogenetic tree of sesquiterpene cyclase homologs STC1 – STC11 from *Fusarium*.

Closely related enzymes are shown in light grey boxes, more distantly related enzymes in dark grey boxes. Enzymes that are likely non-functional due to critical sequence defects are shown in red.

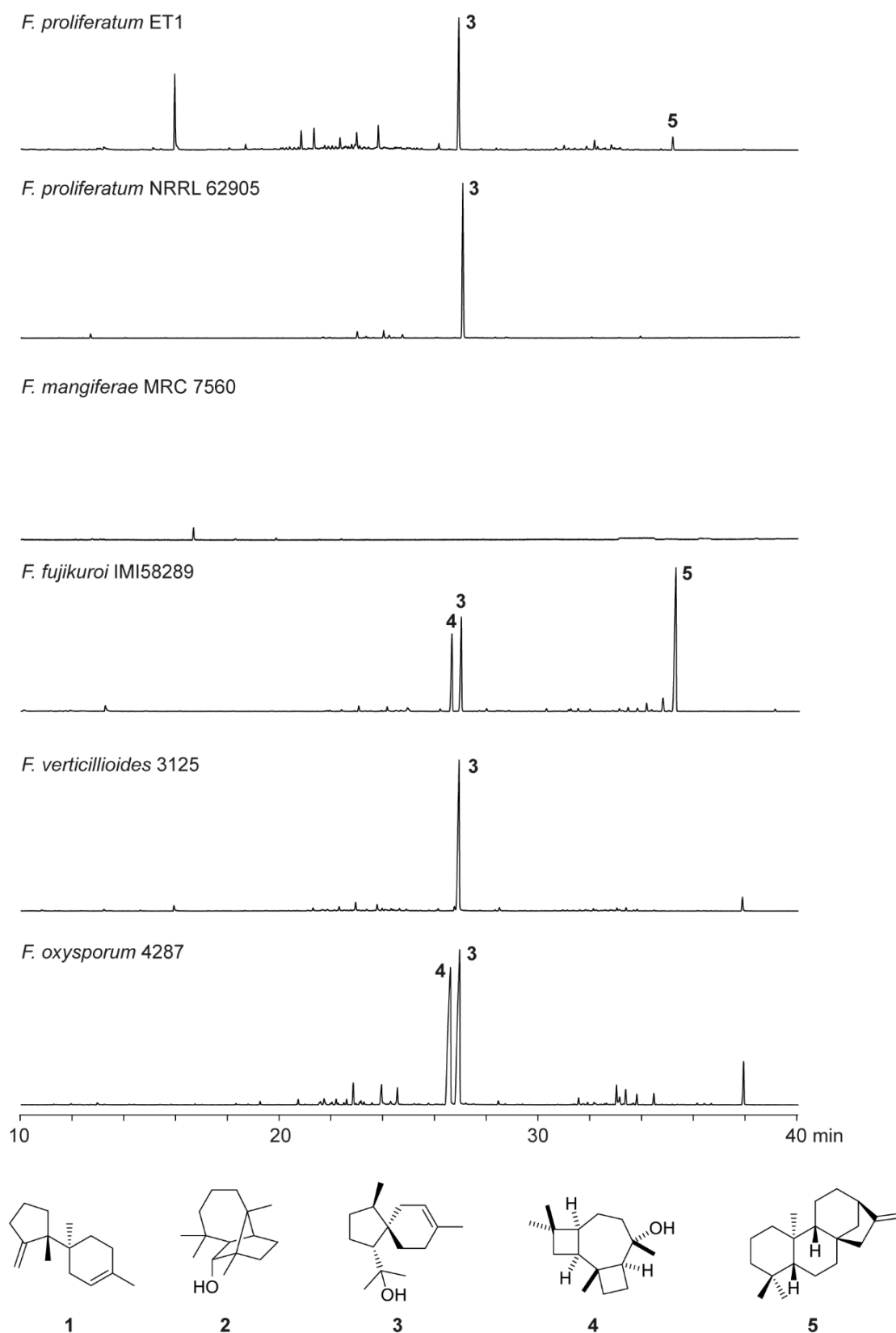


Fig. S6. Total ion chromatograms of headspace extracts from the five *Fusarium* strains of the FFC and *F. oxysporum*.

Structures of identified terpenes are given: 1 - trichodiene, 2 - longiborneol, 3 - (-)- α -acorenol, 4 - (+)-koraïol, 5 - *ent*-kaurene

Alignment STC5

FFUJ_11739 (*Fusarium fujikuroi* IMI58289)
 FPRN_14684 (*Fusarium proliferatum* NRRL 62905)
 FPRO_15464 (*Fusarium proliferatum* ET1)
 FMAN_14887 (*Fusarium mangiferae* MRC 7560)
 EGU79661 (*Fusarium oxysporum* Fo5176)
 FOXG_12225 (*Fusarium oxysporum* 4287)
 EXL40588 (*Fusarium oxysporum* f. sp. *radicis-lycopersici* 26381)
 EWZ33520 (*Fusarium oxysporum* Fo47)
 EY81459 (*Fusarium oxysporum* FOSC 3-a)
 FGSG_08181 (*Fusarium graminearum* PH-1)
 EYB25222 (*Fusarium graminearum* CS3005)

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1	10	20	30	40	50	60
MVKFD-SGSESEMTNGDELHINSKHEVKS	SRMANGNGVHNVPDHDQFQDRAEMEVLILPDL					
MVKFD-SGSESEMTNGDELHINSKHEVKS	SRMANGNGVHNVPDHDQFQDRAEMEVLILPDL					
MVKFD-SGSESEMTNGDDLHINSKHEVKS	SRMANGNGVHNVPDHDQFQDRAEMEVLILPDL					
MVKFD-SGSESEMTNGDEVHINTKHEVKS	SRMANGNGVHNVPDHDQFQDRAEMEVLILPDL					
MVKFD-SGSESEMTNGDELHINAKHEIKPRMANGNGVHNVP	EHQFQDHAEMEVLILPDL					
MVKFD-SGSESEMTNGDELHINAKHEIKPRMANGNGVHNVP	EHQFQDHAEMEVLMLPDL					
MVKFD-SGSESEMTNGDELHINAKHEIKPRMANGNGVHNVP	EHQFQDHAEMEVLMLPDL					
MVKFD-SGSESEMTNGDELHINAKHEIKPRMANGNGVHNVP	EHQFQDHAEMEVLMLPDL					
MVKFD-SGSESEMTNGDELHINAKHEIKPRMANGNGVHNVP	EHQFQDHAEMEVLMLPDL					
MVKFDSSSSESEMTTGDDEVYIDTKHEVKKMKNGNGVYNEPKHQFQGRDEMEVLILPDL						
MVKFDSSSSESEMTTGDDEVYIDTKHEVKKMKNGNGVYNEPKHQFQGRDEMEVLILPDL						
61	70	80	90	100	110	120
FSSLMSVPARENPHYASVKADADEWISFVINADAKWASRNKRVDF	TYLASIWAPDCSAFA					
FSSLMSVPARENPHYASVKADADEWISSVINADAKWASRNKRVDF	TYLASIWAPDCSAFA					
FSSLMSVPARENPHYASVKADADEWISSVINADAKWASRNKRVDF	TYLASIWAPDCSAFA					
FSSLMSVPARENPHYASVKADADEWISSVINADAKWASRNKRVDF	TYLASIWAPDCSAFA					
FSSLMSVPARENPNYASVKAEADEWIASVINADAKWASRNKRVDF	TYLASIWAPDCSAFA					
FSSLMSVPARENPNYASVKAEADEWIASVINADAKWASRNKRVDF	TYLASIWAPDCSAFA					
FSSLMSVPARENPNYASVKAEADEWIASVINADAKWASRNKRVDF	TYLASIWAPDCSAFA					
FSSLMSVPARENPNYASVKAEADEWIASVINADAKWASRNKRVDF	TYLASIWAPDCSAFA					
FSSLMSVPARENPNYASVKAEADEWIASVINADAKWASRNKRVDF	TYLASIWAPDCSAFA					
FSSLMSVPARENPNYASVKAEADDWISSVIKADADWTNRNKRVDFTYLANI	WAPDCSAFA					
FSSLMSVPARENPNYASVKAEADDWISSVIKADADWTNRNKRVDFTYLANI	WAPDCSAFA					
121	130	140	150	160	170	180
LRTSADWNSWAFLFDDQFDEGHLNSNDLEGAINEIARTREIMEGTAPRYTADSEHPIRYVF						
LRTSADWNSWAFLFDDQFDEGHLNSNDLDGAINEIARTREIMEGTAPRYTADSEHPIRYVF						
LRTSADWNSWAFLFDDQFDEGHLNSNDLDGAINEIARTREIMEGTAPRYTADSEHPIRYVF						
LRTSADWNSWAFLFDDQFDEGHLNSNDLDGAINEIARTREIMEGTAPRYTADSEHPIRYVF						
LRTSTDWNSWAFLFDDQFDEGHLNSNDLDGAVNEIARTREIMEGTAPRYTADSEHPIRYVF						
LRTSTDWNSWAFLFDDQFDEGHLNSNDLDGAVNEIARTREIMEGTAPRYTADSEHPIRYVF						
LRTSTDWNSWAFLFDDQFDEGHLNSNDLDGAVNEIARTREIMEGTAPRYTADSEHPIRYVF						
LRTSTDWNSWAFLFDDQFDEGHLNSNDLDGAVNEIARTREIMEGTAPRYTADSEHPIRYVF						
LRTSTDWNSWAFLFDDQFDEGHLNSNDLDGAVNEIARTREIMEGTAPRYTADSEHPIRYVF						
LRTSADWNSWAFLFDDQFDEGHLNSNDLEGAISEIARTREIMEGTAPRYTADSEHPIRYVF						
LQ-----						

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 EYB25222 (*Fusarium graminearum* CS3005)

181	190	200	210	220	230	240
QTLCDRVKQ	NPEGFYAGKPSS	ERFYRRWM	WAHELYWE	GLVAQV	VRTNVEGRS	FTRGPPEEYL
QTLCDRVKQ	SPEGFYAGKPSS	ERFYRRWM	WAHELYWE	GLVAQV	VRTNVEGRS	FTRGPPEEYL
QTLCDRVKQ	SPEGFYAGKPSS	ERFYRRWM	WAHELYWE	GLVAQV	VRTNVEGRS	FTRGPPEEYL
QTLCDRVKQ	SPEGFYAGKPSS	DRFYRRWM	WAHELYWE	GLVAQV	VRTNVEGRS	FTRGPPEEYL
QTLCDRVKQ	SPEGFYAGKPSS	DRFYRRWM	WAHELYWE	GLVAQV	VRTNVEGRS	FTRGPDEYL
QTLCDRVKQ	SPEGFYAGKPSS	DRFYRRWM	WAHELYWE	GLVAQV	VRTNVEGRS	FTRGPDEYL
QTLCDRVKQ	SPEGFYAGKPSS	DRFYRRWM	WAHELYWE	GLVAQV	VRTNVEGRS	FTRGPDEYL
QTLCDRVKQ	SPEGFYAGKPSS	DRFYRRWM	WAHELYWE	GLVAQV	VRTNVEGRS	FTRGPDEYL
QTLCDRVKQ	NPEGFYAGKPSS	DRFYRRWM	WAHELYWE	GLVAQV	VRTNVEGRS	FTRGPDEYL
QTLCDRVKQ	SPEGFYAGKPSS	DRFYRRWM	WAHELYWE	GLVAQV	VRTNVEGRS	FTRGPDEYL
QTLCDRIKQ	SPEGFFIGKPSS	DRFYKRWM	WAHELYWQ	GLVDQV	VRTNVEGRS	YTRRPEEYM
QTLCDRIKQ	SPEGFFIGKPSS	DRFYKRWM	WAHELYWQ	GLVDQV	VRTNVEGRS	YTRRPEEYM

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241	250	260	270	280	290	300
AMRRGSLGAY	PALVNNWAY	GIDLPEE	VADHPLVFEI	MIISDQILLV	KDILSYEKDLRL	
AMRRGSLGAY	PALVNNWAY	GIDLPEE	VADHPLVFEI	MIISDQILLV	NDILSYEKDLRL	
AMRRGSLGAY	PALVNNWAY	GIDLPEE	VADHPLVFEI	MIISDQILLV	NDILSYEKDLRL	
AMRRGSLGAY	PALVNNWAY	GIDLPEE	VADHPLVFEI	MIISDQILLV	NDILSYEKDLRL	
AMRRGSLGAY	PALVNNWAY	GIDLPEE	VADHPLVFEI	MIISDQILLV	NDILSYEKDLRL	
AMRRGSLGAY	PALVNNWAY	GIDLPEE	VADHPLVFEI	MIISDQILLV	NDILSYEKDLRL	
AMRRGSLGAY	PALVNNWAY	GIDLPEE	VADHPLVFEI	MIISDQILLV	NDILSYEKDLRL	
AMRRGSLGAY	PALVNNWAY	GIDLPEE	VADHPLVFEI	MIISDQILLV	NDILSYEKDLRL	
AMRRGSLGAY	PALVNNWAY	GIDLPEE	VADHPLVFEI	MIISDQILLV	NDILSYEKDLRL	
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AMRRGSLGAY	PALVNNWAY	CYGIDLPEE	VADHPSVFEI	MIISDQILLV	NDILSYEKDLRL	
AMRRGSLGAY	PALVNNWAY	CYGIDLPEE	VADHPSVFEI	MIISDQILLV	NDILSYEKDLRL	

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301	310	320	330	340	350	360
GVDHNMVRL	LKAKGLSTQ	QAINEVGVM	INNCYRRYY	RALSELPCF	GEEADRALL	GYLEVE
GVDHNMVRL	LKAKGLSTQ	QAINEVGVM	INNCYRRYY	RALSELPCF	GEEADRALL	GYLEVE
GVDHNMVRL	LKAKGLSTQ	QAINEVGVM	INNCYRRYY	RALSELPCF	GEEADRALL	GYLEVE
GVDHNMVRL	LKAKGLSTQ	QAINEVGVM	INNCYRRYY	RALSELPCF	GEEADRALL	GYLEVE
GVDHNMVRL	LKAKGLSTQ	QAINEVGVM	INDCYRRYY	RALSELPCF	GEEADRALL	GYLEVE
GVDHNMVRL	LKAKGLSTQ	QAINEVGVM	INDCYRRYY	RALSELPCF	GEEADRALL	GYLEVE
GVDHNMVRL	LKAKGLSTQ	QAINEVGVM	INDCYRRYY	RALSELPCF	GEEADRALL	GYLEVE
GVDHNMVRL	LKAKGLSTQ	QAINEVGVM	INDCYRRYY	RALSELPCF	GEEADRALL	GYLEVE
GVDHNMVRL	LKAKGLSTQ	QAINEVGVM	INDCYRRYY	RALSELPCF	GEEADRALL	GYLEVE
GVDHNMVRL	LKAKGLSTQ	QAINEVGVM	INDCYRRYY	RALSKLPCF	GKEADRALL	GFLEVE
GVDHNMVRL	LKAKGLSTQ	QAINEVGVM	INDCYRRYY	RALSKLPCF	GEEADRALL	GFLEVE

	361	370	380	390	400
FFUJ_11739 (<i>Fusarium fujikuroi</i> IMI58289)	KNHALGSL	LSYNTGRYF	SKEDGARVR	KTRELLIPK	KMAAL
FPRN_14684 (<i>Fusarium proliferatum</i> NRRL 62905)	KNHALGSL	LWSYKTGRYF	SKEDGARVR	KTRELLIPK	KMAAL
FPRO_15464 (<i>Fusarium proliferatum</i> ET1)	KNHALGSL	LWSYKTGRYF	SKEDGARVR	KTRELLIPK	KMAAL
FMAN_14887 (<i>Fusarium mangiferae</i> MRC 7560)	KNHALGSL	LWSYKTGRYF	SKEDGARVR	KTRELLIPK	KMAAL
EGU79661 (<i>Fusarium oxysporum</i> Fo5176)	KNHALGSL	LWSYKTGRYF	SKEDGARVR	KTRELLIPK	KMAVL
FOXG_12225 (<i>Fusarium oxysporum</i> 4287)	KNHALGSL	LWSYKTGRYF	SKEDGARVR	KTRELLIPK	KMAVL
EXL40588 (<i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i> 26381)	KNHALGSL	LWSYKTGRYF	SKEDGARVR	KTRELLIPK	KMAVL
EWZ33520 (<i>Fusarium oxysporum</i> Fo47)	KNHALGSL	LWSYKTGRYF	SKEDGARVR	KTRELLIPK	KMAVL
EWY81459 (<i>Fusarium oxysporum</i> FOSC 3-a)	KNHALGSL	LWSYKTGRYF	SKEDGARVR	KTRELLIPK	KMAVL
FGSG_08181 (<i>Fusarium graminearum</i> PH-1)	KNHALGSL	LWSYKTGRYF	SKEDGARVR	KTRELLIPK	SLAAL
EYB25222 (<i>Fusarium graminearum</i> CS3005)	KNHALGSL	LWSYKTGRYF	SKEDGARVR	KTRELLIPK	SLAAL

Alignment STC8

	1	10	20	30	40	50	60
EYB24542 (<i>Fusarium graminearum</i> CS3005)	MGLWLSI	LRLHRGIP	SDKQQTKK	ARATEDSE	DLKSF	IKPLITS	LASELEYSSLTPTD
XP_009261488 (<i>Fusarium pseudograminearum</i> CS3096)	MGIWLSI	LRLHRGIP	SDEQRQTK	KARATADSE	DLKSF	IKPLITS	LASELEYSSLTPTD
FMAN_12837 (<i>Fusarium mangiferae</i> MRC 7560)	MGPTLSS	LSRWSHP	QLRKPVSG	RAERDLEPK	LDLKS	FIEPLITS	FAEEIGYSPLKTTT
EXK29856 (<i>Fusarium oxysporum</i> f. sp. <i>melonis</i> 26406)	MGPTLSS	LSRWSHP	PPPREPV	SGSSERQ	HEPKQDL	RSFIEPLITS	FAEEIGYSPLKTTT
EWY85543 (<i>Fusarium oxysporum</i> FOSC 3-a)	MRPTLSS	LSRWSHP	PPPREPV	SGSSERQ	HEPKQDL	RSFIEPLITS	FAEEIGYSPLKTTT
FVEG_10174 (<i>Fusarium verticillioides</i> 3125)	MGPTLSS	LSRFRWSY	SL-REPVS	GGAKRQ	PEPKQDL	KSFIQPLITS	SFTNEIGYSPLKNTS
FFUJ_09423 (<i>Fusarium fujikuroi</i> IMI58289)	MGPTLSS	SIRSWSHY	QLREPV	SGGAERQ	LEPKQYL	KSFEPLITS	FAEGIGYPLNATS

	61	70	80	90	100	110	120
EYB24542 (<i>Fusarium graminearum</i> CS3005)	NSALWD	AMCLYAD	KTGVQY	DEGTHS	GTGCFK	IGFTYPA	VCFPHHPL
XP_009261488 (<i>Fusarium pseudograminearum</i> CS3096)	NSALWD	AMCLYAD	KTGVQY	DEGTHS	GTGCFK	IGFTYPA	VCFPHHPL
FMAN_12837 (<i>Fusarium mangiferae</i> MRC 7560)	NDALWK	AMRAHAD	SI	GVPEEG	THSWLM	FKVGYV	YPVVC
EXK29856 (<i>Fusarium oxysporum</i> f. sp. <i>melonis</i> 26406)	NVYLWK	AMRIYAD	ST	GVSC	EEGTHS	WLMFKV	GYVYP
EWY85543 (<i>Fusarium oxysporum</i> FOSC 3-a)	NVYLWK	AMRTYAD	ST	GVSC	EEGTHS	WLMFKV	GYVYP
FVEG_10174 (<i>Fusarium verticillioides</i> 3125)	NDVLWK	AMRTHAD	ST	GVPE	EEGTHS	WLMFKV	GYVYP
FFUJ_09423 (<i>Fusarium fujikuroi</i> IMI58289)	NDALWK	TMAHAD	RTD	V	PYEEG	THSWLM	FKVG-VYP

	121	130	140	150	160	170	180
EYB24542 (<i>Fusarium graminearum</i> CS3005)	LDDEAV	TYPDEF	QMFHQ	RFCMGE	KQPL	PLLQGW	ADLMKMA
XP_009261488 (<i>Fusarium pseudograminearum</i> CS3096)	LDDEAV	TYPDEF	QMFHQ	RFCMGE	KQPL	PLLQGW	ADLMKMA
FMAN_12837 (<i>Fusarium mangiferae</i> MRC 7560)	LDDQTV	VDYLEE	FSLFH	ELFCAD	EKQSI	PLLQAW	ADLMKLT
EXK29856 (<i>Fusarium oxysporum</i> f. sp. <i>melonis</i> 26406)	LDDEAA	EYLED	FSMFH	ERFCAD	EKQI	PLLQGW	ADLMKLT
EWY85543 (<i>Fusarium oxysporum</i> FOSC 3-a)	LDDEAA	EYLED	FSMFH	ERFCAD	EKQI	PLLQGW	ADLMKLT
FVEG_10174 (<i>Fusarium verticillioides</i> 3125)	LDDQAA	EYLED	FSMFH	ERFCAD	EKQI	PLLQAW	ADLMKLT
FFUJ_09423 (<i>Fusarium fujikuroi</i> IMI58289)	LDDQAV	VDYHEE	FSMFH	ERFCAD	ENQFI	PLLQAS	ADLMKLT



Fig. S7. Amino acid alignment of two sesquiterpene cyclases, STC5 and STC8, in *Fusarium* spp.

Highly conserved residues for each class of terpene cyclases are yellow, highly conserved aspartate-rich (DDXXD) and NSE triad (NDXXSXXXE) motifs of terpene cyclases that are important for functionality are green, and critical sequence deviations are red.

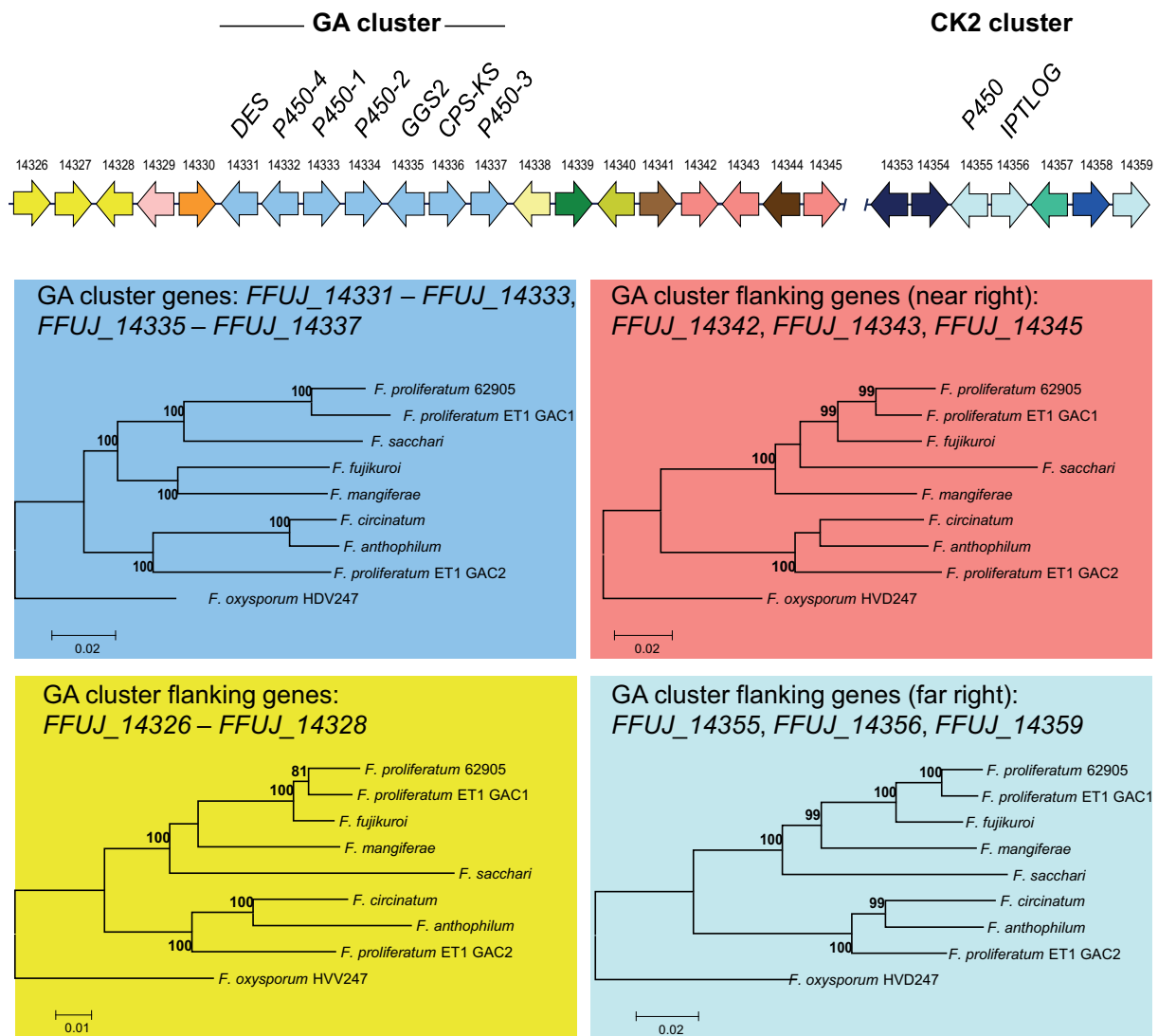


Fig. S8. Maximum likelihood tree inferred from nucleotide sequences of coding regions of GA cluster and GA cluster-flanking genes in FFC members and *F. oxysporum*.

Gene sequences from the GAC2 region of *F. proliferatum* ET1 are more closely related to American-clade sequences (i.e., from *F. anthophilum* and *F. circinatum*) than to other Asian-clade sequences (i.e., from *F. fujikuroi*, *F. mangiferae*, and *F. sacchari* and *F. proliferatum* 62905 and ET1 GAC1). Genes, represented by arrows above the trees, are colour coded to match the coloured boxes around trees. Numbers near branch nodes are bootstrap values.

	IAA precursors			end product
	IPyA	IAM	IAAld	IAA
<i>Mycelium</i>				
<i>Fp_N_WT</i>	14953 ± 3334	8.0 ± 3.7	8826 ± 2943	108.5 ± 48.8
<i>Fp_E_WT</i>	3404 ± 1330	1671 ± 1306	251483 ± 102691	1137 ± 476.2
<i>Fm_WT</i>	163406 ± 61212	185.6 ± 51.2	63015 ± 4608	5871 ± 1745
<i>Ff_WT</i>	5274 ± 1707	157.5 ± 50.0	59537 ± 24522	1099 ± 413.2
<i>Fv_WT</i>	4528 ± 605.0	854.3 ± 139.7	32372 ± 9933	4744 ± 722.6
<i>Fo_WT</i>	23394 ± 10537	14.6 ± 4.9	12005 ± 4565	1533 ± 1001
<i>Fp_E_OE:IAA</i>	62997 ± 4990	11849 ± 295	44829 ± 13028	48863 ± 6409
<i>Fm_OE:IAA</i>	19241 ± 9119	1624 ± 804.5	22837 ± 9264	70721 ± 6295
<i>Ff_OE:IAA</i>	55854 ± 11754	1874 ± 1078	604846 ± 177940	276132 ± 156719
<i>Fv_OE:IAA</i>	6078 ± 1877	5336 ± 2547	50166 ± 27458	119494 ± 63244
<i>Culture filtrates</i>				
<i>Fp_N_WT</i>	6350 ± 3348	<0.1	331131 ± 117879	49236 ± 19306
<i>Fp_E_WT</i>	1824 ± 273.9	<0.1	52640 ± 4370	27273 ± 3876
<i>Fm_WT</i>	210235 ± 17402	<0.1	54243 ± 28752	47114 ± 6606
<i>Ff_WT</i>	7338 ± 741.8	<0.1	577269 ± 58099	179482 ± 17090
<i>Fv_WT</i>	6127 ± 1820	<0.1	94287 ± 27766	42746 ± 12795
<i>Fo_WT</i>	6324 ± 1104	<0.1	49094 ± 1439	13873 ± 6182
<i>Fp_E_OE:IAA</i>	26761 ± 4570	<0.1	766332 ± 107658	63587 ± 17733
<i>Fm_OE:IAA</i>	62105 ± 2897	<0.1	329104 ± 157894	1258106 ± 242179
<i>Ff_OE:IAA</i>	12838 ± 10935	<0.1	2400303 ± 583522	7301405 ± 3334474
<i>Fv_OE:IAA</i>	67940 ± 1047	<0.1	1091454 ± 250751	134337 ± 28538

Fig. S9. Content of IAA and its precursors, produced by mycelium and secreted into the medium.

Cultures of WT and *IAA* and *IAH* overexpressing strains (OE:IAA) were grown in minimal medium for 7 days. Mean values of three biological replicates are given together with standard deviations. Concentrations are in pmol per g of dry weight or 1 L of culture filtrates. IAA, indole-3-acetic acid; IPyA - indolepyruvic acid; IAM - indole-3-acetamide; IAAld, indole-3-acetaldehyde; *Fm*, *Ff*, and *Fv* indicate *F. mangiferae* (Asian clade), *F. fujikuroi* (Asian clade) and *F. verticillioides* (African clade), respectively. *Fp_N* and *Fp_E* indicate *F. proliferatum* strains 62905 and ET1, respectively (Asian clade); *Fo* - *F. oxysporum* (outgroup).

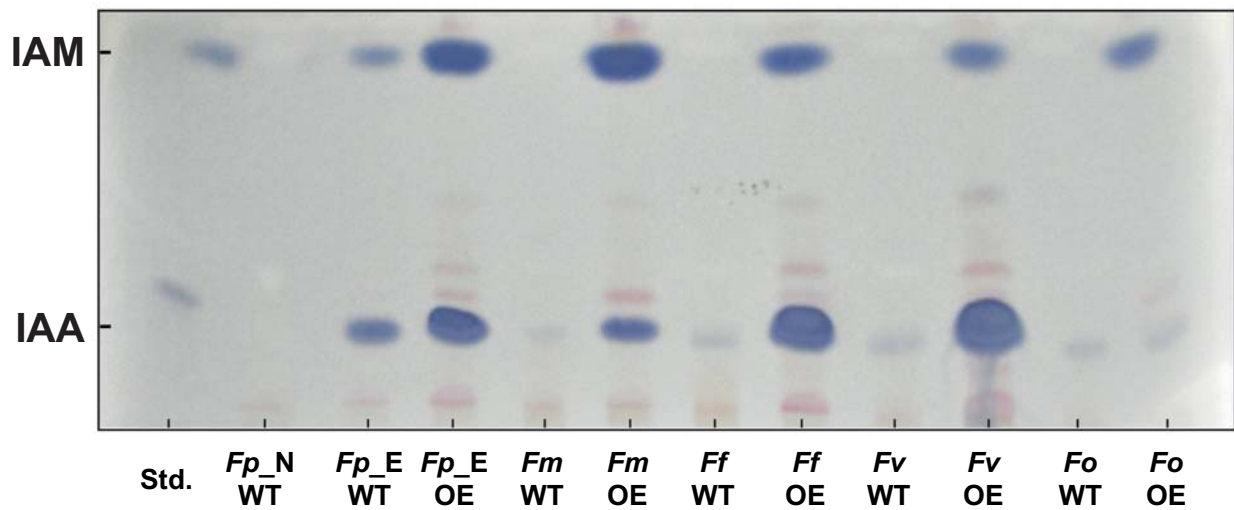


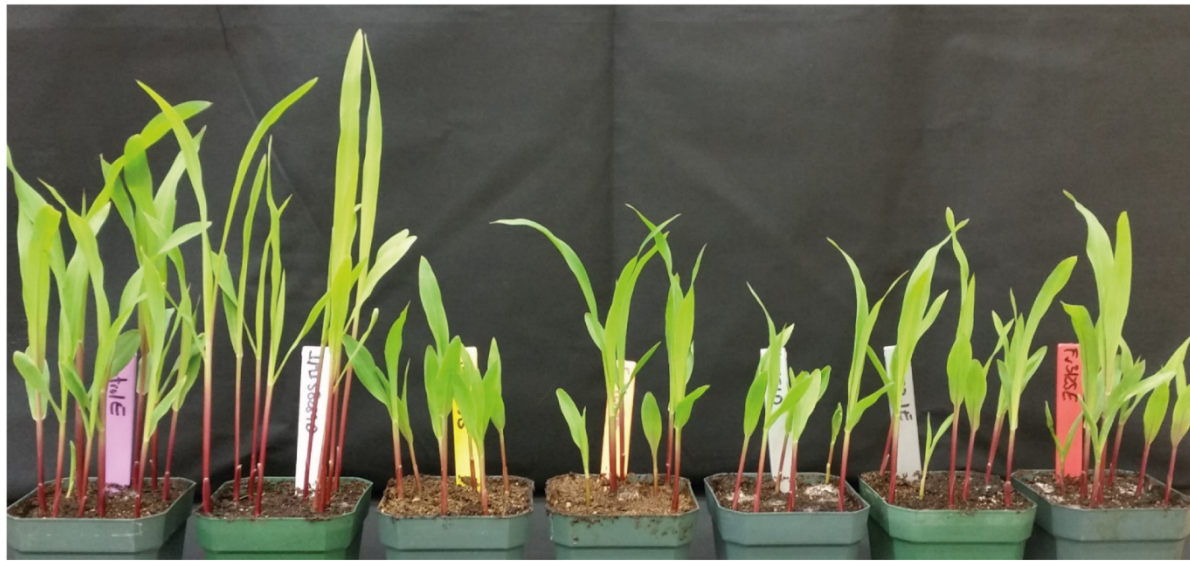
Fig. S10. Thin layer chromatogram of IAM and IAA.

Wild types and *IAAM/IAAH* overexpressing mutant strains were grown under addition of 5mM tryptophan for 48h. Total indoles were extracted from the culture filtrate, and samples were analyzed by TLC. The bands corresponding to IAA and IAM are marked; Std - standard samples of indole-3-acetamide (IAA) and indole-3-acetic acid (IAM), *Fp_N* WT - *Fusarium proliferatum* 62905, *Fp_E* WT - *Fusarium proliferatum* ET1, *Fm* WT - *Fusarium mangiferae* MRC7560, *Ff* WT - *Fusarium fujikuroi* IMI 58289, *Fv* WT - *Fusarium verticillioides* M-3125, *Fo* WT - *Fusarium oxysporum* 4287; OE - overexpression mutants.

	<i>mycelium</i>				<i>culture filtrates</i>			
	iP	tZ	cZ	DHZ	iP	tZ	cZ	DHZ
<i>Fp_N_WT</i>	149 ± 85.6	2.3 ± 2.1	21.6 ± 13.0	0.3 ± 0.2	30.3 ± 15.2	24.8 ± 14.8	509 ± 261	25.4 ± 10.2
<i>Fp_E_WT</i>	25.5 ± 19.5	1.1 ± 0.2	11.5 ± 12.9	0.2 ± 0.2	2.1 ± 1.3	21.4 ± 10.4	58.7 ± 44.7	41.6 ± 23.6
<i>Fm_WT</i>	167 ± 113	2.4 ± 2.2	11.9 ± 8.1	0.2 ± 0.2	77.2 ± 19.6	27.8 ± 5.5	131 ± 67.2	40.0 ± 10.5
<i>Ff_WT</i>	21.4 ± 16.0	2.7 ± 1.9	29.5 ± 33.9	1.3 ± 0.4	24.2 ± 9.2	25.0 ± 7.7	14.9 ± 7.0	60.2 ± 8.8
<i>Fv_WT</i>	33.2 ± 25.7	0.8 ± 0.2	2.8 ± 1.2	0.3 ± 0.2	8.6 ± 3.1	28.2 ± 4.3	65.7 ± 32.5	39.6 ± 20.3
<i>Fo_WT</i>	125 ± 66	5.0 ± 3.2	3.8 ± 0.9	0.6 ± 0.4	50.4 ± 16.9	23.9 ± 7.6	2.1 ± 3.7	38.6 ± 19.3
<i>Fp_E_IL1</i>	1075 ± 58.7	9.2 ± 2.1	2.0 ± 0.4	<0.1	8153 ± 874	2823 ± 125	188 ± 24.5	<0.1
<i>Fp_E_IL2</i>	497 ± 27.5	3.8 ± 1.2	<0.1	<0.1	5513 ± 48.0	753 ± 23.5	63.1 ± 10.9	<0.1
<i>Fp_E_IL1P1</i>	3.9 ± 1.2	437 ± 24.5	106 ± 18.3	1.8 ± 0.2	10.3 ± 1.9	11344 ± 381	102 ± 20.1	56.6 ± 23.7
<i>Fp_E_IL2P2</i>	<0.1	209 ± 10.9	41.7 ± 8.7	0.3 ± 0.3	<0.1	10174 ± 287	78.5 ± 18.8	38.9 ± 9.0
<i>Fm_IL1</i>	6.2 ± 1.2	2.5 ± 1.2	<0.1	<0.1	12.9 ± 2.4	108.5 ± 14.4	29.6 ± 4.2	39.6 ± 7.7
<i>Fm_IL2</i>	<0.1	6.9 ± 2.0	<0.1	<0.1	<0.1	29.1 ± 6.9	9.9 ± 2.8	13.4 ± 3.0
<i>Fm_IL1P1</i>	9.9 ± 2.7	129 ± 24.5	4.3 ± 0.9	<0.1	20.6 ± 2.9	12084 ± 208	389 ± 29.6	1674 ± 93.1
<i>Fm_IL2P2</i>	<0.1	31.5 ± 8.4	<0.1	<0.1	23.8 ± 6.6	695.5 ± 17.9	124 ± 31.5	223 ± 51
<i>Ff_IL1</i>	2184 ± 83.2	62.8 ± 10.0	4.2 ± 1.4	7.8 ± 3.8	49704 ± 2784	14452 ± 398	326 ± 28.6	11.1 ± 0.9
<i>Ff_IL2</i>	71.2 ± 8.8	352 ± 25.9	50.5 ± 9.7	8.5 ± 2.9	19094 ± 792	2092 ± 206	68.9 ± 7.9	2.6 ± 0.7
<i>Ff_IL1P1</i>	835 ± 74.3	216 ± 33.4	34.7 ± 17.2	1.5 ± 0.8	11.3 ± 2.4	27687 ± 479	294 ± 47.0	39.6 ± 5.4
<i>Ff_IL2P2</i>	<0.1	425 ± 50.7	52.5 ± 8.0	7.3 ± 3.8	<0.1	61785 ± 3874	2298 ± 145	192 ± 36.5
<i>Fv_IL1</i>	1874 ± 274	78.6 ± 14.3	2.3 ± 0.5	<0.1	21670 ± 684	1090 ± 156	33.6 ± 7.0	2.1 ± 0.9
<i>Fv_IL2</i>	874 ± 44.8	58.9 ± 18.3	<0.1	<0.1	10304 ± 298	455.5 ± 57.6	34.1 ± 5.9	1.0 ± 0.6
<i>Fv_IL1P1</i>	<0.1	504 ± 28.6	28.9 ± 4.9	0.7 ± 0.3	32.6 ± 7.4	7050 ± 369	502 ± 45.6	42.5 ± 4.7
<i>Fv_IL2P2</i>	<0.1	478 ± 36.5	17.3 ± 3.5	0.3 ± 0.2	<0.1	5612 ± 187	311 ± 28.3	17.2 ± 6.3
<i>Fo_IL1</i>	67.7 ± 10.6	1.7 ± 0.7	<0.1	<0.1	1431 ± 132	209 ± 47.6	19.3 ± 2.9	<0.1
<i>Fo_IL2</i>	135 ± 27.2	3.0 ± 0.4	<0.1	<0.1	5390 ± 261	336 ± 28.9	23.8 ± 4.4	<0.1

Fig. S11. Different forms of cytokinins produced by mycelium and secreted into the medium. Wild type (WT) and *IL* or *ILP* overexpressing mutant strains were grown in minimal medium for 7 days. Mean cytokinin values of three biological replicates are shown together with standard deviations. Concentrations are in pmol per g of dry weight or 1 L of lyophilized culture filtrate. tZ, trans-zeatin; iP, isopentenyladenine; cZ, cis-zeatin; DHZ, dihydrozeatin; Not listed CKs were below the detection limit. *Fp_N* – *F. proliferatum* 62905 (Asian clade); *Fp_E* – *F. proliferatum* ET1 (Asian clade); *Fm* – *F. mangiferae* (Asian clade); *Ff* – *F. fujikuroi* (Asian clade); *Fv* – *F. verticillioides* (African clade); *Fo* – *F. oxysporum* (outgroup); *IL1* – IPTLOG1; *IL2* – IPTLOG2; *IL1P1* – IPTLOG1 and P450-1; *IL2P2* – IPTLOG2 and P450-2.

A



Control

Ff

Fm

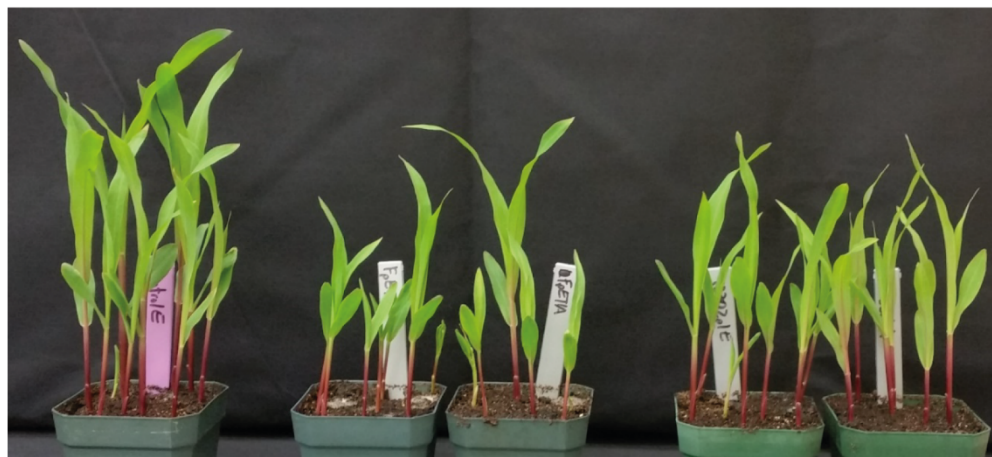
Fo

Fp_E

Fp_N

Fv

B



Control

Fp_E

Fp_N

Fig. S12. Pathotest on maize.

Effects of *Fusarium* strains on maize seed germination and seedling growth. (A) The upper panel shows effects of all species examined: *F. fujikuroi* (*Ff*), *F. mangiferae* (*Fm*), *F. oxysporum* (*Fo*), *F. proliferatum* strains ET1 (*Fp_E*) and 62905 (*Fp_N*), and *F. verticillioides* (*Fv*). Most species except for *F. fujikuroi* caused a reduction in seed germination and reduced seedling growth (B) The lower panel shows that the orchid (*Fp_E*) and maize (*Fp_N*) strains of *F. proliferatum* caused similar effects on maize seedlings.

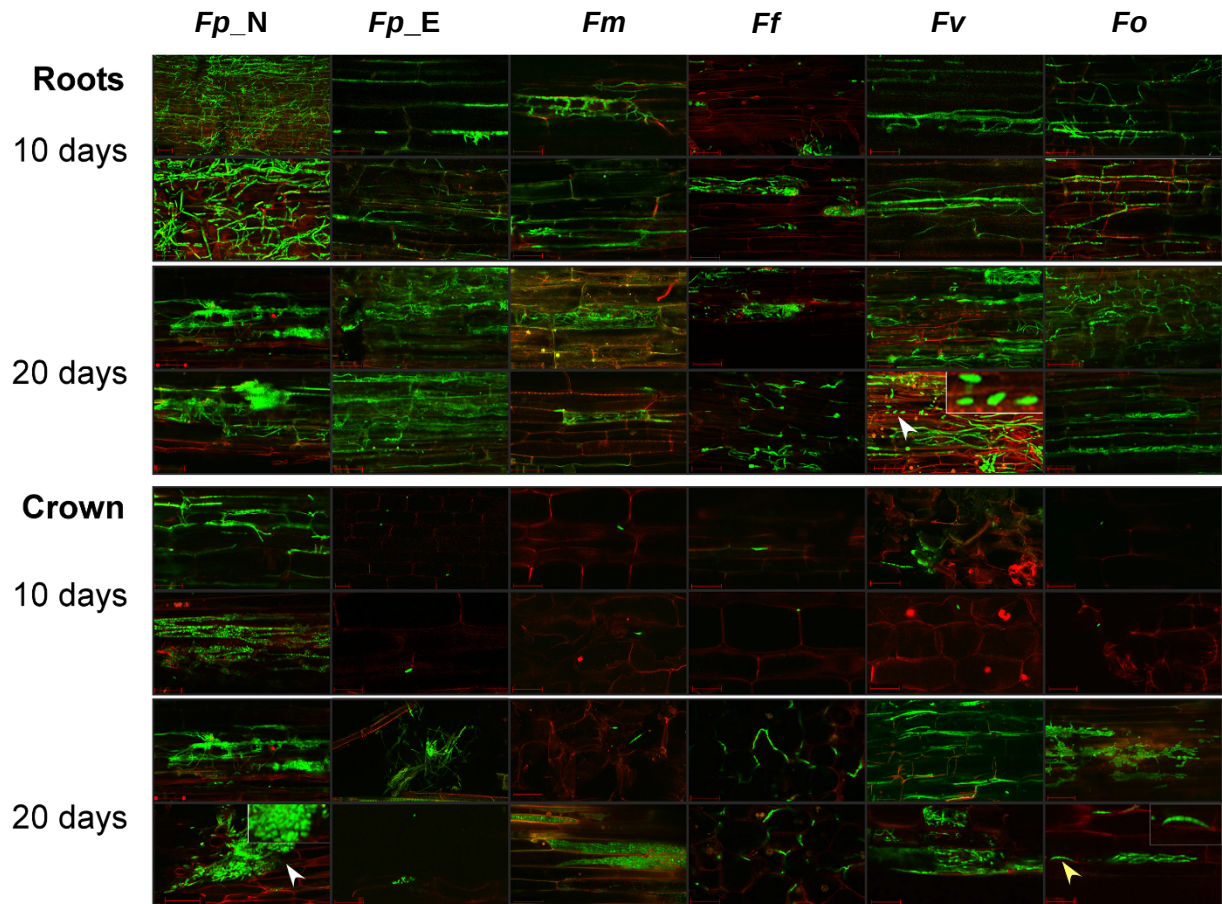


Fig. S13. Microscopic observation of invasive growth of the GFP-tagged *Fusarium* strains. Maize seedlings were grown in soil that was pre-inoculated with GFP-tagged *Fusarium* strains. At 10 and 20 days, tissue samples of root and crown were obtained, stained with propidium iodide (PI) and then scanned by a confocal microscope. For each sample, two representative images are presented. Arrows show location of microconidia (white arrows) in *F. proliferatum* 62905 and *F. verticillioides* and macroconidia (yellow arrows) in *F. oxysporum*. Scale bars = 50 μ m; *F. proliferatum* strains 62905 (*Fp_N*) and ET1 (*Fp_E*), *F. mangiferae* (*Fm*), *F. fujikuroi* (*Ff*), *F. verticillioides* (*Fv*) and *F. oxysporum* (*Fo*).

Supplementary Tables

Table S2. Comparison of the effects of wild-type strains of *Fusarium* on germination of maize seeds and growth of the resulting seedlings.

Species/Treatment	Germination ¹ [%]	Height ¹ [mm]
Control	81 ^a	61.1 ^b
<i>F. proliferatum</i> 62905	61 ^a	49.8 ^b
<i>F. proliferatum</i> ET1	78 ^a	49.7 ^b
<i>F. mangiferae</i>	61 ^a	42.6 ^b
<i>F. fujikuroi</i>	81 ^a	83.8 ^a
<i>F. verticillioides</i>	77 ^a	52.1 ^b
<i>F. oxysporum</i>	73 ^a	50.5 ^b

¹ Values are means from three independent experiments (Table S3). In each experiment, there were five pots per treatment (strain inoculation), and 10 treated seeds were sown per pot. Germination was determined by counting the number of seedlings per pot, dividing by 10, because 10 seeds were sown per pot, and then multiplying by 100. Height was determined by measuring the length of each seedling from the soil line to the tip of the tallest leaf. Data from all three experiments were combined and subjected first to an ANOVA and subsequently to a least squares means test to determine which means were significantly different. Within a column, values followed by different letters are significantly different ($P \leq 0.05$).

Table S3. Summary of results of the pathogenicity assays with wild-type strains of *Fusarium* in the maize seedling assay.

	Percent Seed Germination ¹		Seedling Height [mm] ¹	
Experiment 1				
Strain	Mean	SD	Mean	SD
Control	8.4	1.5	54.8	9.6
<i>F. fujikuroi</i>	7.4	2.3	84.2	22.4
<i>F. mangiferae</i>	5.0	3.3	40.7	22.1
<i>F. oxysporum</i>	8.8	0.8	59.7	19.1
<i>F. proliferatum</i> ET-1	8.6	1.9	53.9	16.6
<i>F. proliferatum</i> 62905	5.6	3.2	48.5	14.6
<i>F. verticillioides</i>	7.4	1.1	56.9	24.0
Experiment 2				
Strain	Mean	SD	Mean	SD
Control	7.4	0.5	62.2	13.2
<i>F. fujikuroi</i>	7.6	1.1	74.7	10.2
<i>F. mangiferae</i>	6.8	2.4	47.0	12.9
<i>F. oxysporum</i>	6.6	2.1	51.8	20.5
<i>F. proliferatum</i> ET-1	8.4	0.9	61.8	10.3
<i>F. proliferatum</i> 62905	5.2	2.4	39.7	22.4
<i>F. verticillioides</i>	8.0	2.0	60.1	11.7
Experiment 3				
Strain	Mean	SD	Mean	SD
Control	8.4	1.9	66.0	21.5
<i>F. fujikuroi</i>	9.4	0.5	92.6	27.4
<i>F. mangiferae</i>	6.6	1.3	39.9	6.3
<i>F. oxysporum</i>	6.6	0.5	47.9	11.2
<i>F. proliferatum</i> ET-1	6.4	1.3	43.0	4.1
<i>F. proliferatum</i> 62905	7.4	2.1	61.3	12.5
<i>F. verticillioides</i>	7.8	0.8	41.5	11.8

¹ Means and standard deviation (SD) values were determined from three independent experiments. In each experiment, there were five pots per treatment (strain), and 10 treated seeds were sown per pot. Percent seed germination was determined at 7 days after planting by dividing the number of seedlings per pot by 10, because 10 seeds were sown per pot, and multiplying by 100. Seedling height was determined by measuring the length of each seedlings from the soil line to the tip of the tallest leaf at 7 days after planting. Seedling weight was determined by pooling all seedling from the same pot and weighing them at 11 days after planting.

Table S5. Primer used in this study.

The name of the primer and its sequence are listed.

Primer	Sequence
Diagnostic primers	
DIAG_IAAM_FW	AGATAACGCTATGTCCGGCC
DIAG_IAAH_FW	TTCCGAGTAGATCGTGACAAC
Primers for quantitative real-time PCR (Fusarium)	
Fver IL1_F	ACGGAGGCGGCACAAC
Fver IL1_R	TCAGGCCAGAAAGGTCAAC
Fver IL2_F	GGGTCTATTTGGCGGATCTTC
Fver IL2_R	CCGCCGTAGACGAGTTTGTAG
Fman IL1_F	ACGGAGGCGGCACAAC
Fman IL1_R	TCAGGCCAGAAAGGTCAAC
Fman IL2_F	CGGGCTATTTGGCGGATCTTCGC
Fman IL2_R	CCGCCGTAGACGAGTTTGTAG
Ffuj IL1_F	GCATCTTTGGTGGTTCGTCAT
Ffuj IL1_R	ACAGACTCTGCCGAGTGACTTG
Ffuj IL2_F	GCTACAGTTTGCGAACGATCAA
Ffuj IL2_R	CCGCCAAATAGACCAACGA
Fman UBI_F	GATCCTCTTGTGCCCCGAGATC
Fman UBI_R	CCGAGCTGTGGCCTCGTA
Ffuj/Fver ACTIN_F	CATTGTCATGTCTGGTGGTACCA
Ffuj/Fver ACTIN_R	AGCAAGGGCAGTGATCTCCTT
Primers for overexpression mutants	
OE_GPD_IAAM_RV	GCGGATAACAATTTACACAGGAAACAGCGGTTAAACGCAGCAGCAAC ATTAC
OE_GPD_IAAM_FW	TACCCCGCTTGAGCAGACATACAATGCGTTTCTTCAGCTTCAACAAC
OE_IAAH_FW	GCGCAGCACAAAACAGCCATGAATTCCAAGCACGCTCTTG
OE_IAAH_RV	TAATCATACATCTTATCTACATACGTTATTCCTGGAAAGCCTGCC
OE_IPTLOG1_FW	CCATCACATCACAATCGATCCAACCATGGAGTCTAACAACCGATTTATG
OE_IPTLOG1_RV	TAATCATACATCTTATCTACATACGCTAGACCCAATCTAGTACACC
OE_IPTLOG2_FW	CCATCACATCACAATCGATCCAACCATGCAATCCAATCAAAAGCTCTG
OE_IPTLOG2_RV	GCAAAGTAGGAATGACATCAGATATCTAATCCCATTCCAGCTCAC
OE_P450IL1_FW2	TACCCCGCTTGAGCAGACATACAATGGCTATCTTACAAGCTCACTTC
OE_P450IL1_RV2	GCGGATAACAATTTACACAGGAAACAGCGGAAATACGGCCTTATACT AGC
OE_P450IL2_FW2	TACCCCGCTTGAGCAGACATACAATGTATCCCACTTCACTGGCC
OE_P450IL2_RV2	GCGGATAACAATTTACACAGGAAACAGCGGACGTATGGGTCTAATCTT AAAC
Primers for sequencing	

IAAH_SEQ_FW	ACTGTACAGCATCGAAGATGAC
P450IL1_SEQ_RV	GAATAAACTTGTCGGCATGCG
P450IL2_SEQ_RV	GCCAGGTTAATTTGACAAGGTAC
IAAM_SEQ_RV	GGCGAAGTGGGAGTTGAAG
IAAH_SEQ_RV	GTCATCGGCTGAGAAGATTTGG
P450IL1_SEQ_FW	ATTCAAACAGCTCTCCTTGTAC
P450IL2_SEQ_FW	ATAGCTCTCCGTGATTAC
cDNA check	
CHECK_IAAM_FW	CGTTGGCTTGAATCGATCAGAC
CHECK_IAAM_RV	CAGTAACGTTAAGTGGATCCTC
CHECK_IAAH_FW	CTGCTTTCTTCTCTCATCAACAC
CHECK_IAAH_RV	AGTGAAGGTGGTTCAGGTGC