

## Supplementary data for

### “Getting ready for host invasion: elevated expression and action of xyloglucan endotransglucosylases/hydrolases in developing haustoria of the holoparasitic angiosperm *Cuscuta*”

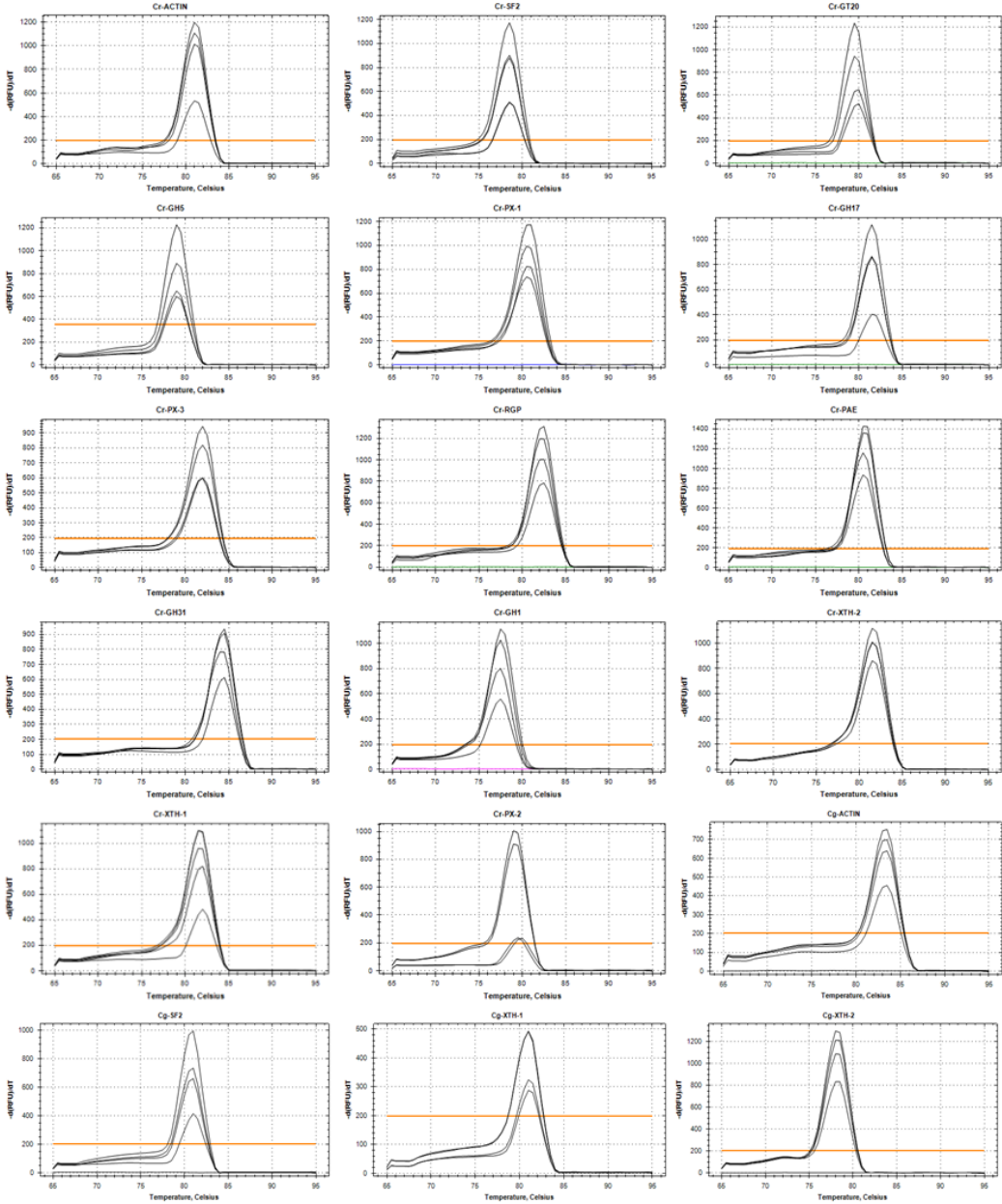
Stian Olsen, Bernd Striberny, Julien Hollmann, Rainer Schwacke, Zoë Popper and Kirsten Krause

Supplementary Table S1. Sequences of gene-specific primers with respective amplicon sizes and PCR efficiencies.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Amplicon size	Efficiency	R <sup>2</sup>
<i>Cr-ACTIN</i>	ATGGAAGCTGCTGGAATCCAC	TTGCTCATACGGTCAGCGATG	140 bp	96.3 %	0.999
<i>Cr-SF2</i>	CGAGGATTTGTTTTACAAGTATGG	CGACCACGAATAGCGTCTTCC	126 bp	102.3 %	0.998
<i>Cr-GH1</i>	TGGTATGGGGTATGCCTCTC	AACCAGCCAAACACGAAATC	82 bp	105.7 %	0.991
<i>Cr-GH31</i>	TCGGTCCACGACCTTTTAC	TTGCAGTCCAAACAGCAGAC	147 bp	105.9 %	0.996
<i>Cr-GH17</i>	CAAGTCTTTTGTCCCACCAA	CGAACACCCTTTTCCACTACA	144 bp	99.6 %	0.990
<i>Cr-XTH-1</i>	CACTTCCAACACTGGGTCCT	AATCCGCCCAAAGAGGTA	226 bp	90.0 %	0.992
<i>Cr-XTH-2</i>	ATAGTCATGGAAGCCGGTTG	GCTTGTGGGAGGAGACTCTG	208 bp	102.4 %	0.998
<i>Cr-PAE</i>	TGTTTAGCCGATGCTGGTTAC	AGCCTTACACGAAGCAGACAA	129 bp	107.8 %	0.997
<i>Cr-RGP</i>	CCAACGAAAATCATCCGAGT	GTTCTTGATGTGCTGCTCCA	237 bp	103.0 %	0.999
<i>Cr-PX-1</i>	TTGGCCTCGGAGTTAATGAC	GGATCCACGTTTGTCTCGTT	109 bp	102.3 %	1.000
<i>Cr-PX-2</i>	ACCCACCAATGTGACCATT	CTTGGGAGAGGAGATGCAAG	141 bp	100.7 %	0.999
<i>Cr-PX-3</i>	CCACCCGTGACGTTATCTCT	TGGAAGGATGGCTTTGGTAG	121 bp	102.9 %	0.996
<i>Cr-GH5</i>	AATAACCACGGATGGTTGGA	GCTGATGCTGTGCTTGGTTA	137 bp	95.6 %	0.993
<i>Cr-GT20</i>	CACCGAAGCAGACCAATACAT	CCGAGATTATACCCGGAACAT	241 bp	103.7 %	0.995
<i>Cg-ACTIN</i>	CCCATACCAACCATCACTCC	TGAGGATATTCAGCCCTTG	138 bp	105.1 %	0.996
<i>Cg-SF2</i>	ACATCGTCTCTCAGGGAAA	CATCAGCATCACGAGCATCT	205 bp	108.9 %	0.988
<i>Cg-XTH-1</i>	AGATGATGTGTTGGGGCTTC	TGGTGAAGCCATACACTCTGC	137 bp	95.0 %	0.987
<i>Cg-XTH-2</i>	TAAGGCCAGATACCCACAG	AAACATGAGCCCAATTCCAC	133 bp	102.4 %	0.997

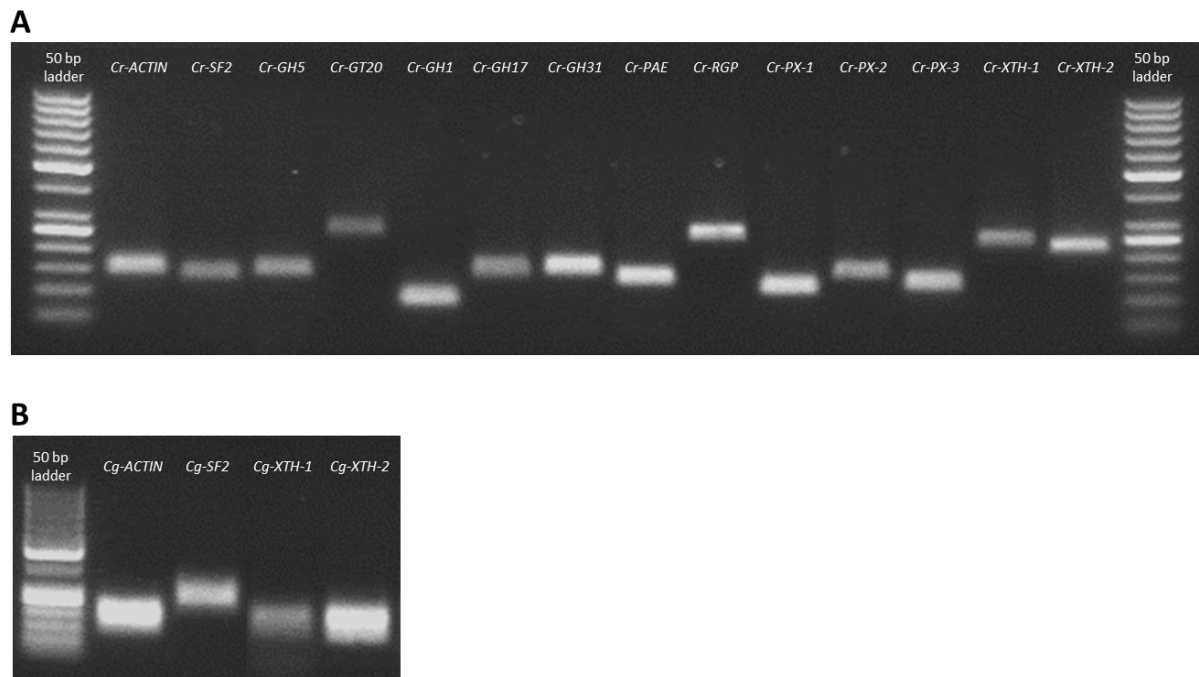
Supplementary Tables S2 and S3 are presented in a separate Excel file.

Supplementary Figure S1



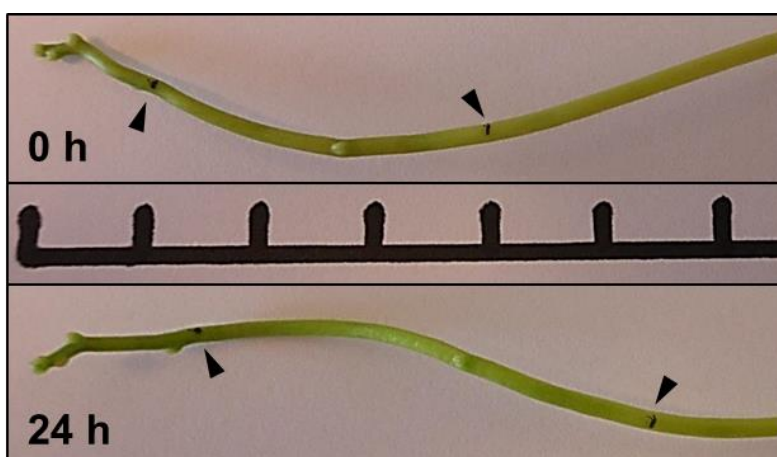
Suppl. Fig. S1. Melt peaks of qPCR amplicons. The negative derivative of the change in fluorescence intensity is plotted as a function of temperature.

## Supplementary Figure S2



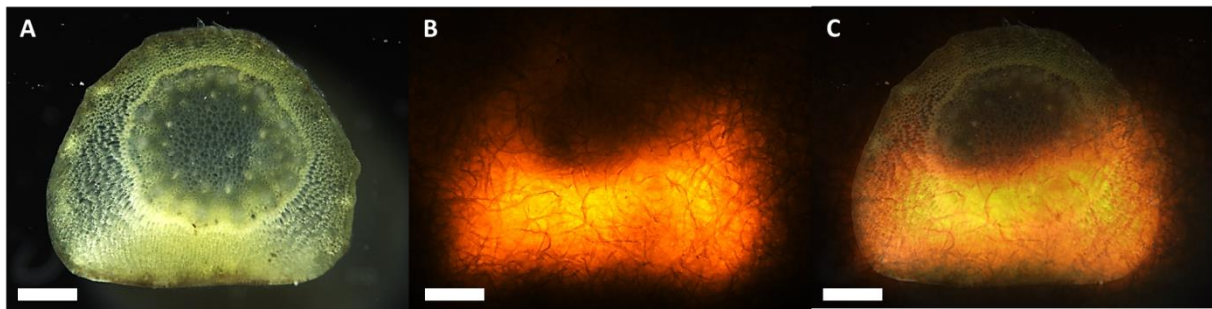
Suppl. Fig. S2. Size separation of (A) *C. reflexa* and (B) *C. gronovii* qPCR amplicons on 2 % agarose gels. GeneRuler 50 bp Ladder (Life Technologies) is used for size estimation.

## Supplementary Figure S3



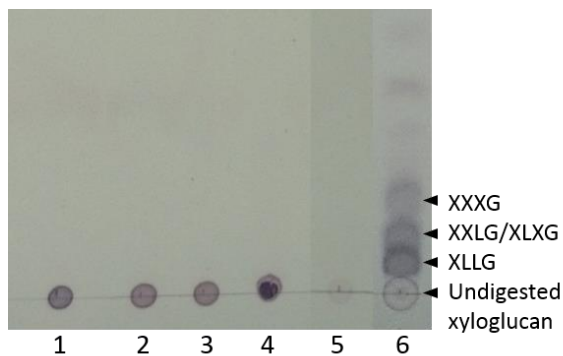
Suppl. Fig. S3. Stem elongation occurring in the region just below the apical shoot tip as indicated by increased distance between markings (arrowheads) after 24 hours. Distances between ruler marks are one cm.

### Supplementary Figure S4



Suppl. Fig. S4. XET action in FR light-induced haustoria. (A) Cross-section of haustorium that was tissue printed on XET test paper three days after light treatment. (B) Fluorescence micrograph showing XET action in the printed tissue in A. (C) Merged picture of A-B. Scale bars are 500  $\mu\text{m}$ . Note that the vein pattern in B and C comes from the fibres in the test paper.

### Supplementary Figure S5



Suppl. Fig. S5. Hydrolytic activity of a haustorial enzyme extract from *C. reflexa* towards xyloglucan. Enzymes were extracted from *C. reflexa* haustoria by homogenizing plant material in extraction buffer (50 mM Na-acetate pH 5.5, 300 mM NaCl, 10 % glycerol, 10 % PVP40) and incubating on ice for two hours. Thin-layer chromatography of 2  $\mu\text{l}$  sample-spots was executed on silica gel with n-butanol:acetic acid:water (3:1:1) as mobile phase. Carbohydrates on the chromatogram were detected by staining with the thymol-sulfuric acid reagent. Lane 1: tamarind xyloglucan only; Lanes 2, 3 and 4: incubation of tamarind xyloglucan with *C. reflexa* enzyme extract for zero, two and 24 hours at 25  $^{\circ}\text{C}$ , respectively; Lane 5: enzyme extract only; Lane 6: incubation of tamarind xyloglucan with a recombinant xyloglucanase (E-XEGP from Megazyme) at 25  $^{\circ}\text{C}$ . The four main xyloglucan oligosaccharide (XXXG, XXLG, XLXG and XLLG) hydrolysis products are marked with arrow tips.