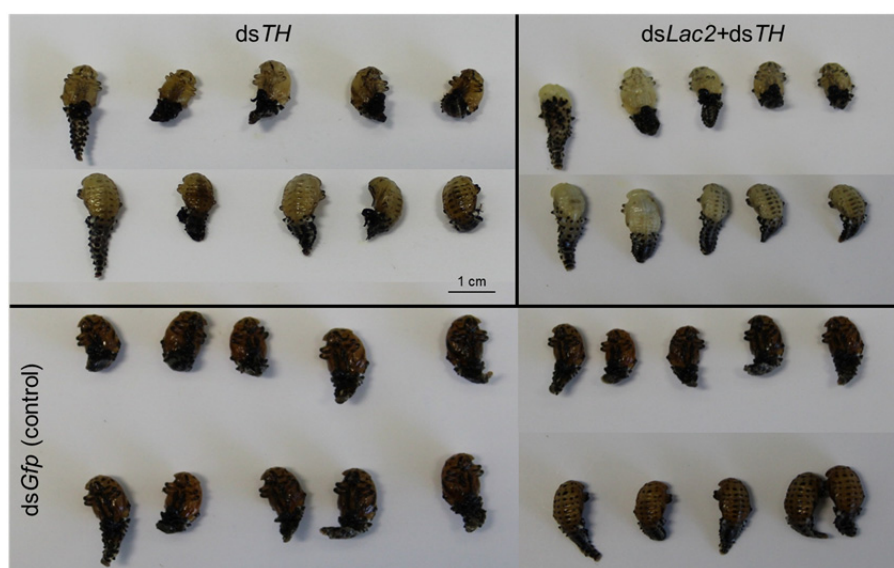
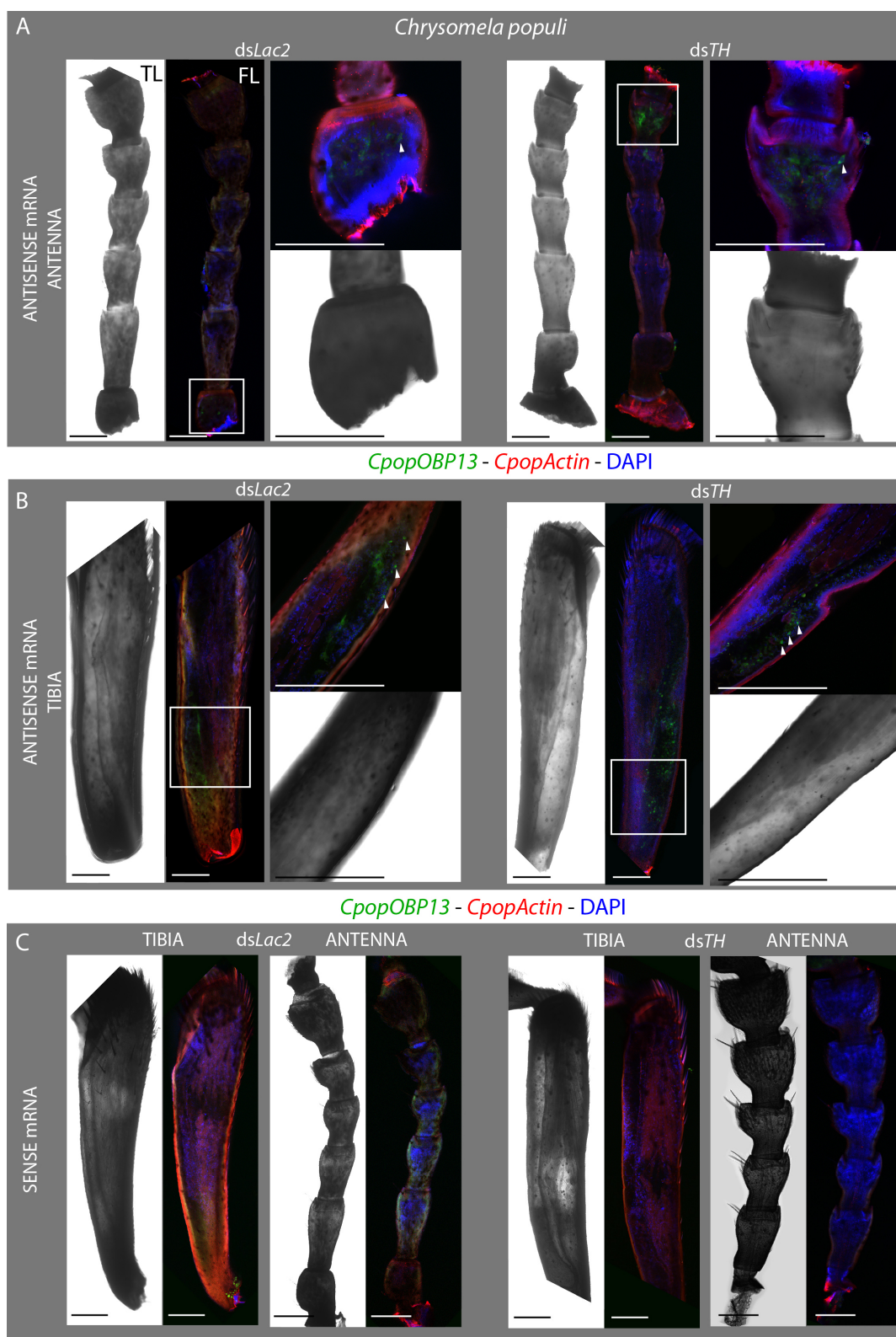


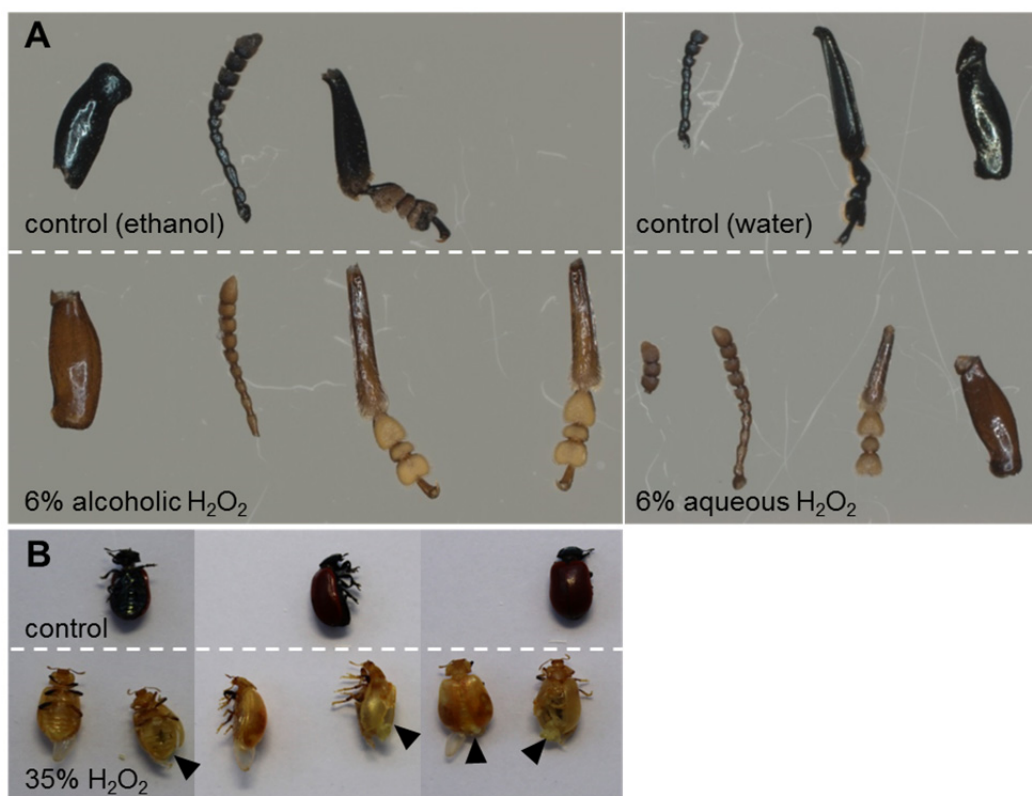
**Fig. S1. A:** Expression of *laccase2* (*Lac2*) or *tyrosine hydroxylase* (*TH*) as RNAi targets in untreated developmental stages and adult tissues/organs of *Chrysomela populi* as analyzed by qPCR. N=4 biological replicates, except larva, pupa and wings (N=3). **B:** Expression of the gustatory receptor *CpopGR1* and odorant-binding protein *CpopOBP13* as RNA FISH targets in untreated tissues/organs of adult *C. populi* beetles as analyzed by qPCR. N=6 biological replicates. Bars represent means $\pm$ s.e.m.



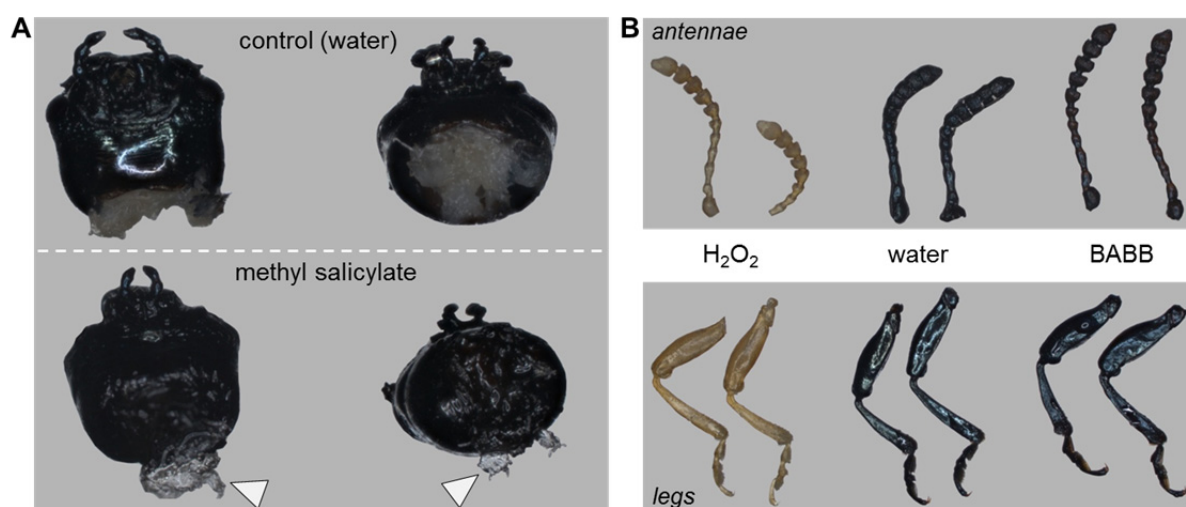
**Fig. S2.** Young *C. populi* pupae (3 days old) were injected dsRNA targeting *TH* or *Lac2* + *TH* combined. RNAi-based clearing of cuticular pigmentation was evident in both cases already in late pupae (10 days old) in comparison to darker RNAi-controls (*dsGfp* injection).



**Fig. S3.** Clearing of cuticular pigmentation by RNAi enables FISH in whole-mount appendages of *C. populi*. **A:** Antennae and **B:** tibiae from *Lac2* or *TH*-silenced individuals with biotin-labelled antisense mRNA of the odorant-binding protein *CpopOBP13* (green fluorescence) in combination with digoxigenin-labelled antisense mRNA for *CpopActin* (red) as well as nuclear staining via DAPI (blue). **C:** Using sense mRNA of *OBP13* as negative control in both RNAi-cleared appendages did not result in distinct green fluorescence, whereas positive controls (antisense *CpopActin* and DAPI) resulted in similar fluorescent signals as in A and B. Arrowheads indicate exemplary stained cells. Proximal parts of the organs are at the bottom, and distal part at the top of the image. TL - transmitted light microscopy (light panels), FL - fluorescence image from confocal laser scanning microscopy (dark panels). N=3 biological replicates for each treatment. Scale bar represents 200  $\mu$ m.



**Fig. S4. A:** Incubation of antennae and bipartite legs from *C. populi* beetles for three weeks in 6% alcoholic or aqueous H<sub>2</sub>O<sub>2</sub> did clear pigmentation in comparison to control incubations (ethanol or water), but did degrade 63.7% of total RNA (see also Fig. 2D). **B:** Incubation of whole beetles in 35% H<sub>2</sub>O<sub>2</sub> for five days resulted in cuticular decoloration (in comparison to water control incubations), which was however partly incomplete, especially on the legs, while also wings became damaged (arrowheads). Two biological replicates per treatment were analyzed.



**Fig. S5.** Incubation of chemosensory appendages of *C. populi* beetles in **A:** methyl salicylate for one week did clear soft tissue such as the brain (arrowheads), but not the cuticle of the head; **B:** BAAB (1:2 benzyl alcohol/benzyl benzoate) incubation for three weeks did not clear cuticular pigmentation of antennae or legs in contrast to incubation in 35% H<sub>2</sub>O<sub>2</sub> as control. Two biological replicates per treatment were analyzed.

**Table S1. Primer sequences used for qRT-PCR, RNAi and RNA FISH.** Cpop – *C. populi*; Pco – *Phaedon cochleariae*. T7 promoter sequence in italics. eIF4A – eukaryotic initiation factor 4 alpha; EF1 – eukaryotic elongation factor 1; Lac2 – laccase2; TH – tyrosine hydroxylase; GR – gustatory receptor; OBP – odorant binding protein; fw – forward; rev – reverse.

Primer name	
qRT-PCR:	5'-3'- sequence
CpopelF4A_fw	TTTGTAATACCCGCCGCAAG
CpopelF4A_rev	TCCATGCATCGCAGAAACAG
CpopEF1 $\alpha$ _fw	TCATCGGTCACGTAGATTCTGG
CpopEF1 $\alpha$ _rev	TTTCGATGGTACGCTTGTCG
CpopLac2q_fw	CTCGTTTTCTGGAGAGAGATAC
CpopLac2q_rev	CCAATCCTCTGAGTTGTATCC
CpopTHq_fw	CTAATGACTCCAGTGTAGAACC
CpopTHq_rev	GACGACTTCTTCTCTGTGAG
CpopGR1q_fw	AGCTCCAGACAGTTCCTTTC
CpopGR1q_rev	AGATCTGGGTTGATGTCTTCGAG
CpopOBP13q_fw	GCCAAGACCAAGAAGGGAGAAT
CpopOBP13q_rev	GATTTTGCCTTGTGCGTCCATG
RNAi:	
CpopLac2_mai_fw	<i>TAATACGACTCACTATAGGGAACGCTGGTACCCACTTCTG</i>
CpopLac2_mai_rev	<i>TAATACGACTCACTATAGGGGTAACGCCACACTCTCCCAA</i>
PcoLac2_mai_fw	<i>TAATACGACTCACTATAGGGACGGCAAAGGACAATTCAGG</i>
PcoLac2_mai_rev	<i>TAATACGACTCACTATAGGGAGGCCTGGGTTGCAAAAATTC</i>
CpopTH_mai_fw	<i>TAATACGACTCACTATAGGGTCAACGCGCAGTATTGGTCT</i>
CpopTH_mai_rev	<i>TAATACGACTCACTATAGGGGAACACCTTGCGGTACTCCA</i>
PcoTH_mai_fw	<i>TAATACGACTCACTATAGGGACAAGGAATACCGTGCCAGG</i>
PcoTH_mai_rev	<i>TAATACGACTCACTATAGGGCCGAACCTCGACAGTGAACCA</i>
RNA FISH:	
CpopGR1_fish_fw	ATGTTCACTCGGATATTGGCGTT
CpopGR1_fish_rev	TCAGTTCCTAGGTATCTTCTGTATGTGA
CpopOBP13_fish_fw	ATGTCTCCTTACAGTGCCAGTCCTGCAT
CpopOBP13_fish_rev	TTAAACAAGGCTGAGATGTGAGGGGG
CpopActin_fish_fw	ATGTGTGACGATGATGTAGCGGC
CpopActin_fish_rev	TTAGAAGCACTTGCGGTGGACG