METHODS

Cuscuta was initially obtained from a tomato field in California, and seed stocks were derived from self-pollination through several generations in the Westwood laboratory. The isolate was initially previously identified as *Cuscuta pentagona*. *C. pentagona* is very closely related to *C. campestris*, and the two are distinguished by microscopic differences in floral morphology; because of this they have often been confused[29]. We subsequently determined that our isolate is indeed *C. campestris*. *A. thaliana sgs2-1* mutants[30] were a gift from H. Vaucheret (INRA Versailles). *A. thaliana dcl4-2t* mutants (GABI_160G05[31]) were obtained from the *Arabidopsis* Biological Resource Center (Ohio State University). *A. thaliana seor* mutants (GABI-KAT 609F04[18]) were a gift from M. Knoblauch (Washington State University). The *A. thaliana tir1-1/afb2-* and *afb3-4* mutants[32] were a gift from G. Monshausen (Pennsylvania State University). The *bik1* mutant[16] was a gift from T. Mengiste (Purdue University). The *scz2* mutant[22] was a gift from R. Heidstra (Wageningen University). All *A. thaliana* mutants were on the Col-0 background.

Growth conditions and RNA extractions. For initial experiments (small-RNA-seq and RNA blots in Fig. 1), *A. thaliana* (Col-0) plants were grown in a growth room at 18–20 °C with 12 h light per day, illuminated (200 μmol $m^{-2}s^{-1}$) with metal-halide (400 W, GE multi-vapour lamp) and spot-gro (65 W, Sylvania) lamps. *C. campestris* seeds were scarified in concentrated sulfuric acid for 45 min, rinsed 5–6 times with distilled water and dried. The seeds were placed in potting medium at the base of four-week-old *A. thaliana* seedlings and allowed to germinate and attach to hosts. The *C. campestris* plants were allowed to grow and spread on host plants for an additional three weeks to generate a supply of uniform shoots for use in the experiment. Sections of *C. campestris* shoot tip (~10 cm long) were placed on the floral stems of a fresh set of *A. thaliana* plants. Parasite shoots coiled around the host stems and formed haustorial connections. Tissues from plants that had established *C. campestris* with at least two coils around healthy host stems and clear parasite growth were used in these studies. Control plants were grown under the same conditions as parasitized plants, but were not exposed to *C. campestris*.

For the preparation of tissue-specific small-RNA libraries, tissues were harvested after *C. campestris* cuttings had formed active haustorial connections to the host. This was evidenced by growth of the *C. campestris* shoot to a length of at least 10 cm beyond the region of host attachment (7–10 days after infection). Three tissues were harvested from the *A. thaliana*–*C. campestris* associations: 2.5 cm of *A. thaliana* stem above the region of attachment, *A. thaliana* and *C. campestris* stems in the region of attachment (referred to as the interface), 2.5 cm of the parasite stem near the point of attachment. To remove any possible cross-contamination between *A. thaliana* and *C. campestris*, harvested regions of the parasite and host stem were taken 1 cm away from the interface region and the surface of each harvested tissue cleaned by immersion for 5 min in 70% ethanol, the ethanol was decanted and replaced, the process was repeated three times and the stems were blotted dry with a Kimwipe after the final rinse. All three sections of tissue were harvested at the same time, and material from 20 attachments was pooled for small-RNA extraction. Small RNA was extracted from ~100 mg of each tissue using the mirPremier microRNA Isolation Kit (Sigma-Aldrich) according to the manufacturer's protocol. Small RNA was analysed using an small-RNA kit (Agilent) on a 2100 Bioanalyzer platform.

Samples used for 5′-RLM-RACE (Fig. 2d) and qRT–PCR (Fig. 3a) analyses of *A. thaliana* targets were prepared as described above with the following modifications: Col-0 *A. thaliana* hosts were cultivated in a growth room with 16-h days, 8-h nights, at ~23 °C under cool-white-fluorescent lamps. Attachment of *C. campestris* cuttings was promoted by illumination with far-red LED lighting for 3–5 days, and total RNA was extracted using Tri-reagent (Sigma) per the manufacturer's suggestions, followed by a second sodium-acetate–ethanol precipitation and wash step. Samples used for RNA blots of secondary siRNA accumulation from *A. thaliana* mutants and replicate small-RNA-seq libraries were obtained similarly, except that the samples were derived from the primary attachments of *C. campestris* seedlings on the hosts instead of from cuttings. In these experiments, scarified *C. campestris* seedlings were first germinated on moistened paper towels for three days at ~28 °C, then placed adjacent to the host plants with their radicles submerged in a water-filled 0.125-ml tube.

C. campestris pre-haustoria (Extended Data Fig. 7) were obtained by scarifying, germinating and placing seedlings as described above, next to bamboo stakes in soil, under illumination from cool-white fluorescent lights and far-red-emitting LEDs. Seedlings coiled and produced pre-haustoria four days after being placed, and were harvested and used for total-RNA extraction (used for RNA blots in Fig. 4b) using Tri-reagent as described above. *N. benthamiana* was grown in a growth room with 16-h days, 8-h nights, at ~23 °C, under cool-white fluorescent lamps. Three-to-four-week-old plants served as hosts for scarified and germinated *C. campestris* seedlings. Attachments were promoted by three-to-six days with supplementation by far-red-emitting LEDs. Under these conditions, *C. campestris* attached to the petioles, and not the stems, of the *N. benthamiana* hosts. Interfaces and control petioles from un-parasitized hosts were collected seven-to-eight days after successful attachments, and total RNA (used for RNA blots in Fig. 4b and small-RNA-seq libraries) was recovered using Tri-reagent as described above.

Small-RNA-seq. The initial small-RNA-seq libraries were constructed using the Tru-Seq small-RNA kit (Illumina) per the manufacturer's protocol and sequenced on an HiSeq2500 instrument (Illumina). Subsequent small-RNA-seq libraries (replicate two using *A. thaliana* hosts, and the *N. benthamiana* experiments) used the NEBnext small-RNA library kit (New England Biolabs), following the manufacturer's instructions. Raw small-RNA-seq reads were trimmed to remove 3′-adapters and filtered for quality and trimmed length ≥16 nucleotides using cutadapt[33] version 1.9.1 with the settings "-a TGGAATTCTCGGGTGCCAAGG–discard-untrimmed -m 16–max-n = 0". For experiments where *A. thaliana* was the host, trimmed reads that aligned with zero or one mismatch (using bowtie[34] version 1.1.2, settings "-v 1") to the *A. thaliana* plastid genome, the *Cuscuta gronovii* plastid genome (*C. gronovii* was the closest relative to *C. campestris* for which a completed plastid-genome assembly was publicly available), *A. thaliana* rRNAs, tRNAs, small nuclear RNAs (snRNAs), and small nucleolar RNAs (snoRNAs) were removed. Similarly, for experiments where *N. benthamiana* was the host, the reads were cleaned against the *C. gronovii* plastid genome, the *N. tabacum* plastid genome and rRNAs, and a set of tRNAs predicted from the *N. benthamiana* genome using tRNAscanSE.

For the original *A. thaliana* host data, the clean reads were aligned and analysed with reference to the combined TAIR10 *A. thaliana* reference genome and a preliminary version 0.1 draft genome assembly of *C. campestris* using ShortStack[35] (version 3.8.3) with default settings. The resulting annotated small-RNA loci (Supplementary Data 1) were analysed for differential expression (interface versus parasite stem) using DESeq2[36], with a $\log_2$ fold threshold of 1, alternative hypothesis of 'greaterAbs', and alpha of 0.05. *P* values were adjusted for multiple testing using the Benjamini–Hochberg procedure, and loci with an adjusted *P* value of ≤0.05 (equivalent to an FDR of ≤0.05) were denoted upregulated in interfaces relative to parasite stem. Among the upregulated loci, those annotated by ShortStack as miRNAs deriving from the *C. campestris* genome which produced either a 21- or 22-nucleotide mature miRNA (Supplementary Data 2) were retained and further analysed. The predicted secondary structures and observed small-RNA-seq read coverage was visualized (Supplementary Data 3, 4) using strucVis (version 0.3; https://github.com/MikeAxtell/strucVis).

For analysis of mRNA-derived secondary siRNAs, the clean small-RNA-seq reads from the original *A. thaliana* experiment were aligned to the combined TAIR10 representative cDNAs from *A. thaliana* and our preliminary version 0.1 transcriptome assembly for *C. campestris*, using ShortStack[35] v3.8.3, with the settings –mismatches 0,–nohp, and defining the full length of each mRNA as a locus using the option –locifile. The resulting counts of small-RNA alignments for each mRNA were used for differential-expression analysis, comparing interface to host stem, using DESeq2[36] as described above. *A. thaliana* mRNAs with significantly upregulated (FDR ≤0.05) small RNAs in the interface compared to host stem were retained for further analysis. The cDNA sequences of these loci were retrieved and used for miRNA target predictions using GSTAr (v1.0; https://github.com/MikeAxtell/GSTAr); the full set of mature miRNA and miRNA* (Supplementary Data 2) from the interface-induced *C. campestris* miRNA loci were used as queries.

Analysis of the second set of *A. thaliana*–*C. campestris* small-RNA-seq data aligned the cleaned reads to the combined *A. thaliana* and *C. campestris* reference genomes as described above, except that the list of loci derived in the analysis of the original data (Supplementary Data 1) was used as a –locifile in the ShortStack analysis. Differential-expression analysis was then performed using DESeq2 as described above. Analysis of the *N. benthamiana*–*C. campestris* small-RNA-seq data began with a ShortStack analysis of the cleaned reads against the combined *N. benthamiana* (v0.4.4) genome and the preliminary assembly of the *C. campestris* genome, using default settings. The *de novo N. benthamiana* loci obtained from this run were retained. The resulting alignments were used to quantify abundance of small RNAs from the *C. campestris* small-RNA loci defined with the original data. The resulting read counts were then used for differential-expression analysis with DESeq2 as described above. Analysis of secondary siRNAs derived from *N. benthamiana* mRNAs was performed in a similar way to the *A. thaliana* mRNA analysis described above, except that the combined transcriptomes were from *C. campestris* and *N. benthamiana* (v0.4.4 annotations).

RNA blots. Small RNA gel blots were performed as previously described[37] with modifications. For the blots shown in Fig. 1b, 1.8 μg of small RNA from each sample was separated on 15% TBE–Urea Precast gels (Bio-Rad), transblotted onto the Hybond NX membrane and cross-linked using 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide[38]. Hybridization was carried out in 5× SSC, 2× Denhardt's solution, 20 mM sodium phosphate (pH 7.2), 7% SDS with 100 μg ml^{-1} salmon testes DNA (Sigma-Aldrich). Probe labelling, hybridization and washing were performed as described[37]. Radioactive signals were detected using Typhoon FLA