Electronic supplementary information (ESI)

ESI, Fig. S1† Binding of eGFP-THA and eGFP-LCI to soybean leaves of different type and age. eGFP-THA, eGFP-LCI, or eGFP (all at 750 nM) were sprayed onto the first trifolium of 2-, 3-, or 4-wk-old plants or onto the second trifolium or primary leaf of 4-wk-old plants. After drying off, treated leaves were thoroughly rinsed with water and analyzed for absence or presence of green fluorescence using confocal microscopy. (Scale bar: 250 µm).

ESI, Fig. S2† In vitro antifungal activity of DS01, THA, and DS01-THA. Uredospores of
P. pachyrhizi were germinated for 18 h at saturated humidity on glass slides that had been covered with polyethylene foil in the absence (water) or presence of 10 µM of the indicated peptide (DS01, THA, or DS01-THA). To exclude unspecific inhibition of spore germination, peptides were digested with proteinase K (final concentration 100 µg/mL) for 1 h at 60 °C before they were added to the *P. pachyrhizi* spores. Developing appressoria were counted in a microscope (n > 100). Shown are means ± SD. Stars indicate significant differences (****p < 0.0001) in paired *t*-test between spores incubated with water or the indicated peptides.

ESI, Fig. S3† Electron transport rate of soybean leaves upon treatment with DS01-THA. Leaves of 2-wk-old soybean plants were sprayed with an aqueous solution of DS01-THA (50 µM). Treatment with water served as a negative and application of the photosynthetic activity-impairing fungicide Maneb (0.3%) as a positive control. Electron transport rate was determined at 1 dpa by pulse-amplitude modulated chlorophyll fluorometry combined with saturating pulse analysis of fluorescence quenching. Data are presented as boxplots with boxes spanning the IQR and whiskers extending to 1.5*IQR (Tukey boxplot). Stars indicate significant differences (****p < 0.0001) between plants treated with water or Maneb in paired *t*-test. n = 32. ETR, electron transport rate.

ESI, Fig. S4† THA-DS01 dipeptide inhibits *C. graminicola* development. GFP-expressing *C. graminicola* was grown on oatmeal agar (50 g/L oatmeal, 15 g/L agar) and kept in UV light at 24 °C and 16-hr light / 8-hr dark cycle. Every second week overgrown agar plugs were transferred to fresh plates to keep the fungus. *C.*

graminicola conidia were harvested by rinsing a 2-wk-old overgrown agar plate with 2 mL deionized water supplemented with 0.02 % Tween 20. The conidia suspension was transferred into a THA-DS01 (final concentration 50 µM) solution. Final condia density was 5 x 10^6/mL. Condia in water served as a control. Droplets of 10 µL were placed on detached corn leaves and incubated for up to 4 days. (A) 2 d after inoculation, appressoria were seen on control leaves (black dots). No appressoria were formed in the presence of THA-DS01. (B) 1 and 4 d after inoculation conidia germination and mycelial growth was examined. Neither germination nor fungal growth in the corn leaf was seen when the condia were suspended in THA-DS01 solution before. Green fluorescence of GFP-expressing *C. graminicola* was recorded using fluorescence microscopy. In A, “+” indicates presence whereas “-“ denotes absence of THA-DS01.

ESI, Fig. S5† Peptide purification. eGFP and eGFP-anchor peptide fusions were purified by affinity chromatography and separated on a gel for quality control. 1, eGFP (31.5 kDa); 2, eGFP-LCI (37.0 kDa); 3, eGFP-THA (33.9 kDa); M, PageRuler™ Prestained Protein Ladder (Thermo Scientific).