The *Fusarium oxysporum* Avr2-Six5 effector pair alters plasmodesmatal exclusion selectivity facilitating cell-to-cell movement of Avr2.

Lingxue Cao, Mila C. Blekemolen, Nico Tintor, Ben J.C. Cornelissen and Frank L. W. Takken

Molecular Plant Pathology, Swammerdam Institute for Life Science, University of Amsterdam, Amsterdam, 1098 XH, Netherlands

*Correspondence: Frank L.W. Takken (f.l.w.takken@uva.nl)

**Running title:** The Avr2/Six5 effector pair alters plasmodesmata

**Supplemental Figures**
Supplemental Figure 1. Avr2 and Six5 co-localize in the cytosol and nucleus of agro-infiltrated *N. benthamiana* cells. GFP-$\Delta$spSIX5, RFP-$\Delta$spAVR2, or RFP-$\Delta$spAVR2/GFP-$\Delta$spSIX5 were expressed in *N. benthamiana* leaves using agro-infiltration, and fluorescence was monitored using confocal microscopy. In GFP-$\Delta$spSIX5/ RFP-$\Delta$spAVR2 expressing *N. benthamiana* leaf epidermal cells fluorescence was observed in the cytosol and nucleus Yellow fluorescence shows the co-localization of Avr2 and Six5 in cytosol and nucleus following co-infiltration of both strains.
Figure S2. Avr2 and Six5 co-localize and interact at plasmodesmata and in the cytoplasm. Confocal images of *N. benthamiana* leaf Agro-infiltrated with BiFC constructs of VYN::ΔspSIX5 and SCYC::ΔspAVR2. Green fluorescence resulting from the interaction of VYN-ΔspSix5 and SCYC-ΔspAvr2 was observed in the cytosol (a cytoplasmic strand is marked with a star) and at spots at the cell periphery. The green spots co-localize with aniline blue stained plasmodesmata-associated callose. Chloroplasts show green auto-fluorescence.
Figure S3. Cell-to-cell movement assay to assess Six5-GFP translocation. (A) Agro-infiltrated *N. benthamiana* leaves co-expressing mCherry-HDEL and SIX5-GFP with either 35S:ΔspAVR2 or 35S:GUS. Fluorescence was analyzed 72 hours after infiltration using confocal microscopy. Transformed cells show both a red ER-localized signal and a green Six5-GFP signal localized to the cytoplasm and nucleus (indicated by *). The ER-localized mCherry is unable to move to neighboring untransformed cells and serves as a marker for
transformation. No red or green fluorescence is observed in untransformed cells adjacent to transformed cells in presence or absence of Avr2. (B) To assess translocation of Six5-GFP protoplasts were isolated from ΔspSIX5-GFP/mCherry-HDEL agro-infiltrated leaves and the percentage of green, red and green/red fluorescent cells were determined using flow cytometry. Auto-fluorescence levels were established by measuring untransformed cells (mock). Based on the mock four quadrants could be distinguished following expression of the ΔspSIX5-GFP/mCherry-HDEL construct: Q1; non-transformed cells, Q2; mCherry-only, Q3; mCherry + GFP and Q4; GFP-only expressing cells. In both presence and absence of Avr2 zero to few GFP-only cells were found in Q4. Note that the overall GFP signal intensity is about 10 fold lower than with Avr2-GFP. (C) No significant difference in GFP only cells is detected between presence or absence of AVR2. Two independent biological replicates were performed, one of which is shown here consisting of 3 infiltrated leafs and subsequent count of 50,000 cells per leaf.