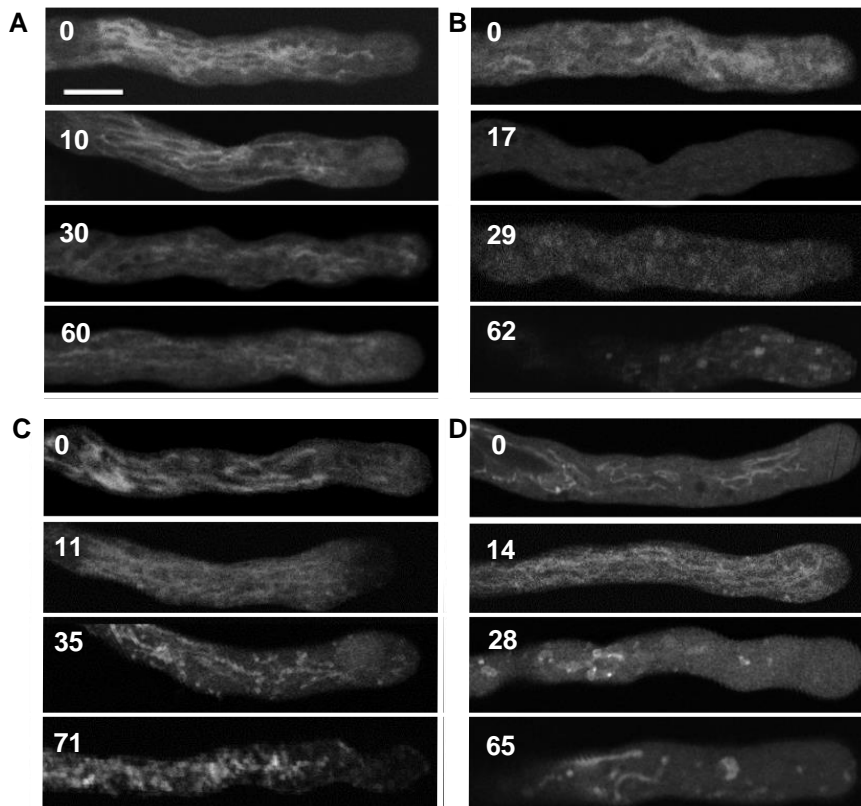


Wilkins et al. **Supplemental Figure 6. Artificial manipulation of cytosolic pH of pollen tube triggers alterations in vacuolar organization.**



Supplemental Figure 6. Artificial manipulation of cytosolic pH of pollen tube triggers alterations in vacuolar organization

Papaver pollen tubes were: (A) untreated (incubated in growth medium) or treated with (B) 50 mM propionic acid pH 5.5 to mimic SI-induced acidification, (C) pre-treated with 50 mM propionic acid pH 7 for 10 min prior to addition of PrsS, or (D) 50 mM propionic acid pH 7. The vacuole was then labelled with c-DCFDA. Scale bar, 10 μ m.

(A) The vacuole of untreated pollen tubes at 0, 10, 30, and 60 minutes did not undergo any reorganization or disruption of the vacuole. (B) Pollen tubes treated with propionic acid pH 5.5, underwent vacuole reorganization within 17 min, with no tubular structure visible. By 62 min only small spots of vacuolar signal remained. This provides evidence that acidification of the cytosol has an extreme effect on the organization and the stability of the vacuole in *Papaver* pollen tubes, and suggests that acidification of the cytosol may play a role in triggering vacuolar breakdown. (C) SI-induced pollen pre-treated with propionic acid pH 7 for 10 minutes underwent some reorganization within the first 11 min and the typical reticulate structure was not as well defined. At 35 min the vacuole underwent major reorganization and aggregated into bundles. Further aggregation continued thereafter, and at 71 min of treatment there were only pockets of vacuolar signal remaining, indicating that it had not completely broken down. Blocking SI-induced acidification with propionic acid pH 7 results in vacuolar reorganization but not vacuolar breakdown. (D) Pollen tubes treated with propionic acid pH 7 showed slight reorganization of the vacuolar signal at 14 min; the typical reticulate structure was not so well defined and had moved into the pollen tube tip. The vacuole continued to reorganize into tight bundles of vacuolar signal, but did not appear to undergo vacuolar breakdown.