Supplementary Material

Host-released dimethylsulphide activates the dinoflagellate parasitoid *Parvilucifera sinerae*

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Materials and Methods

Host and parasitoid strains and culture maintenance

Experiments were conducted with strains of *Alexandrium minutum* (AMP4 and AMP13), *Karlodinium veneficum* (K24) and *Amphidinium carterae* (ACRN03) of the culture collection of the Centro Oceanográfico de Vigo (CCVIEO), Spain, and the culture collection of the Institut Ciències del Mar, Barcelona, Spain. Non-axenic culture stocks were grown in L1 medium (Guillard, 1995) at 20±1ºC, 90 µmol photons m^{-2} s^{-1} and a 12:12 hour photoperiod.

The culture of the *Parvilucifera sinerae* parasitoid (strain ICMB 852; Garcés and Hoppenrath, 2010) was propagated by transferring aliquots of mature sporangia (1 mL) every 5–7 days into an uninfected host stock culture of *A. minutum* AMP4 in sterile polystyrene Petri dishes. The time needed for the formation of a mature sporangium in the *A. minutum* culture at 20ºC is 5 days, but asynchrony was observed in the formation and maturation of sporangia after induced infection. To assure complete and synchronic maturation of all the sporangia population before the experiments, sporangia were kept at 4ºC in the dark. In the range of 12-15 days, all infected cells were in the mature sporangium stage (i.e., the stage at which sporangia are completely full of dormant zoospores, and there are no host cells left). Samples

were used for experiments always within this 12-15 days period after infection. Therefore, the stage of the sporangia was always the same.

Mature sporangia were transferred from 4ºC to fresh medium at 20±1°C in the light one day before experiments were conducted. No significant opening of sporangia was observed with medium only, i.e., in the absence of host cells or exudates or DMS solution.

Experiments and sporangia activation counts

For chemical signalling experiments, the *A. minutum* AMP4 culture in exponential growth was diluted with L1 medium to a concentration that, once mixed with the sporangia in the experimental chambers, gave a concentration of 5000 host cells mL^{-1}. Exudates were prepared by filtering 10 mL of the culture through 0.22-µm pore size Swinnex filters (Millipore) right before the experiment. Exudates of *A. minutum* AMP13 were prepared in the same way as AMP4. In the case of *A. carterae* and *K. veneficum* exudates, they were prepared from cultures with total cell biovolumes equivalent to the 5000 cells mL^{-1} of *A. minutum*.

Experimental mixtures were prepared in triplicate 2-mL phytoplankton chambers by pipetting aliquots of 0.5 mL of mature *P. sinerae* sporangia stock at 20ºC, with no host cells, and adding 1.5-mL aliquots of potential host, exudates, chemical solutions or control medium. Initial *P. sinerae* sporangia and host concentrations in the chambers were 100-1000 sporangia mL^{-1} and 5000 host cells mL^{-1}, respectively. *P. sinerae* activation rates were determined in simultaneous triplicates by counting mature inactive (full) sporangia every 5 minutes during 30 minutes, and every 10 minutes until completing 60 minutes, under a Leica–Leitz DM IRB inverted microscope, a Leica–Leitz DM IL inverted microscope and a Nikon DIAPHOT inverted microscope, respectively. Data were normalized to the initial concentrations of inactive sporangia, and activation rate constants were calculated as the slope of the logarithm-converted numbers over the first 30 minutes. Negative controls consisted of L1 medium additions to the parasitoid suspensions.

Chemical solutions and analyses

Stock solutions of dimethylsulphoniopropionate (DMSP·HCl, TCI America) and acrylate ($C_3H_3O_2Na$, Aldrich) were prepared by weighing and dissolution in MilliQ water. The stock solution of DMS was prepared by adding a pellet of NaOH to 10 mL of the stock solution of

DMSP; the base hydrolyzed DMSP into equimolar amounts of dimethylsulphide (DMS) and acrylate. A few µL of the stock solutions were added to L1 medium immediately before the addition to the *P. sinerae* suspension.

DMS concentrations were measured in GF/F-filtered aliquots of exudates by a purge and trap gas chromatographic method described elsewhere (Galí *et al*., 2011). DMSP concentrations in exudates were measured after alkaline hydrolysis into DMS.

References

Galí M, Saló V, Almeda R, Calbet A, Simó R. (2011). Stimulation of gross dimethylsulfide (DMS) production by solar radiation. *Geophys Res Lett* **38**: L15612, doi:10.1029/2011GL048051.

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Guillard RRL. (1995). Culture Methods. In: Hallegraeff GM, Anderson DM, Cembella AD (eds) *Manual on Harmful Marine Microalgae*. IOC Manuals and Guides., UNESCO, Vol 33, pp 551.

Movie S1. Video recording of the last stages of the infection cycle of *A. minutum* by *P. sinerae* that includes the activation of the flagellate zoospores and their release through opened opercula in the sporangium wall. Images were recorded at 200-400 magnification with a Marlin F080C camera (Allied Vision Technology) attached to a Nikon DIAPHOT 200 inverted microscope.