Crash agent model systems for crop protection.

Pest Models: Understanding Pest Life Cycles and Infection Mechanisms Panel, Barriers to Scale:

Algae Crop Protection Workshop

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April 21, 2021

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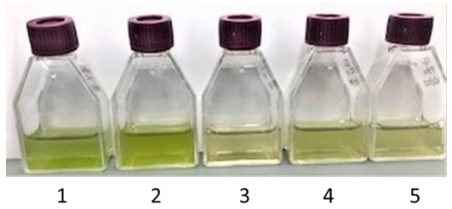
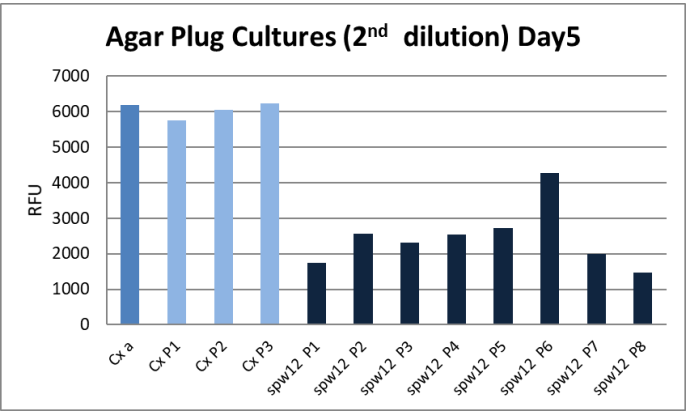
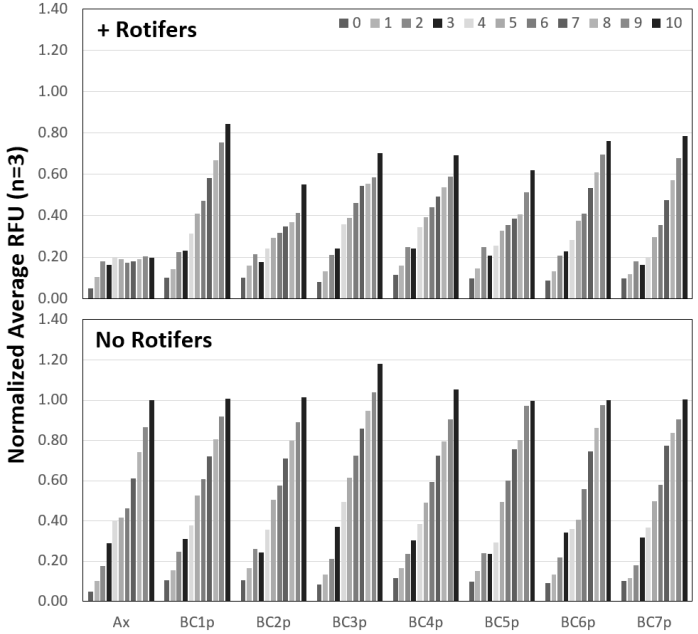
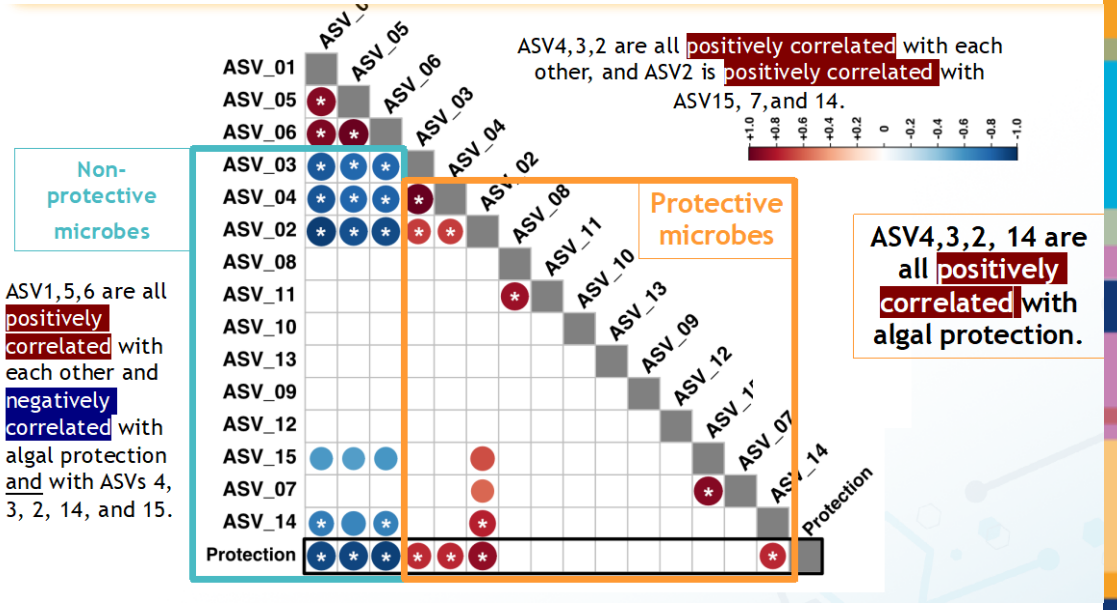
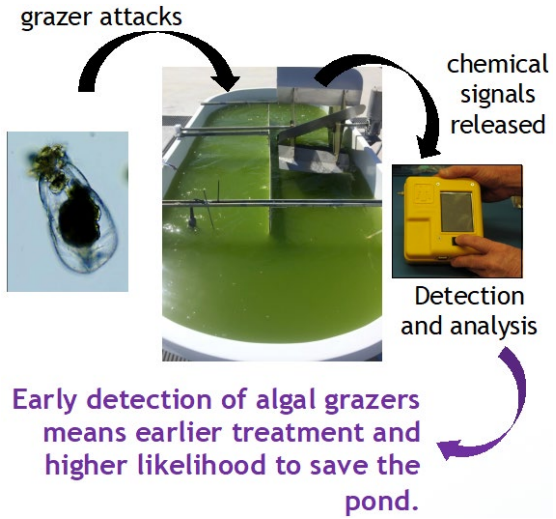
Crop Protection Studies at SNL: Identification, Detection, Characterization, & Countermeasures

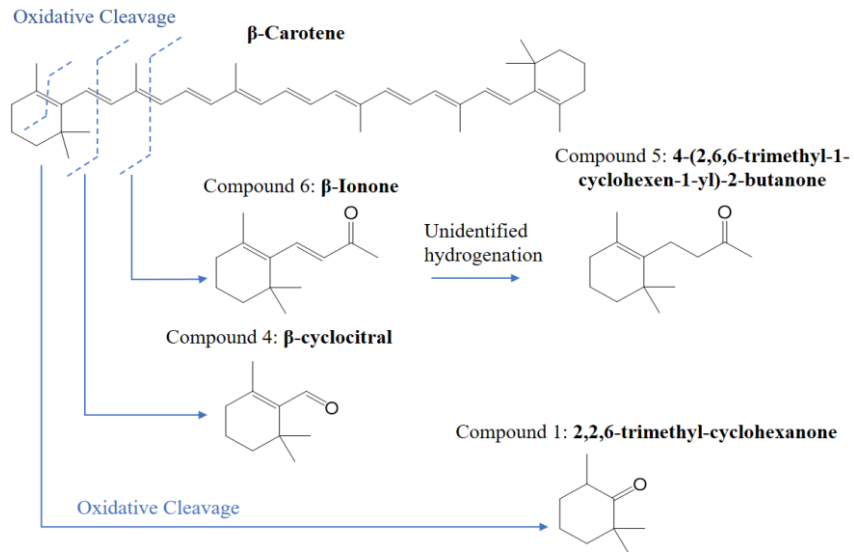


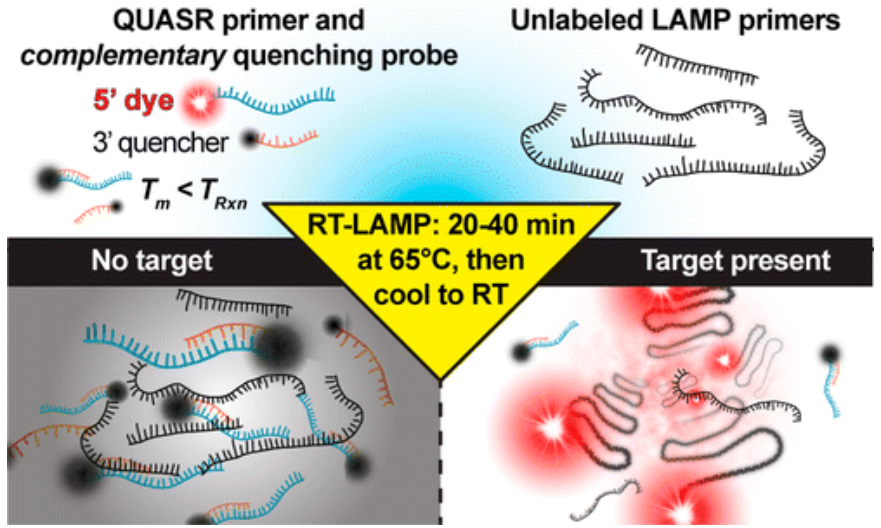












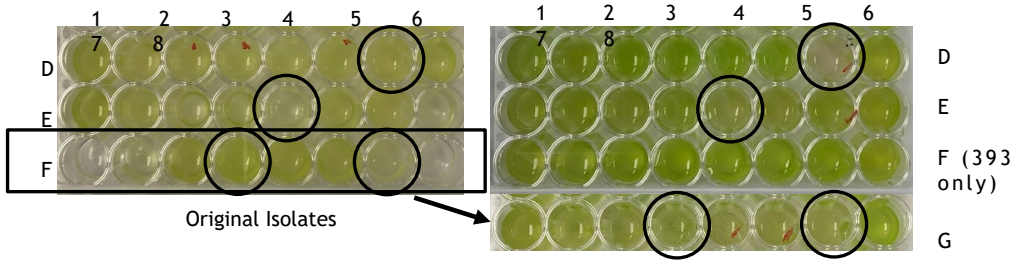
Two types of model systems: applied and basic

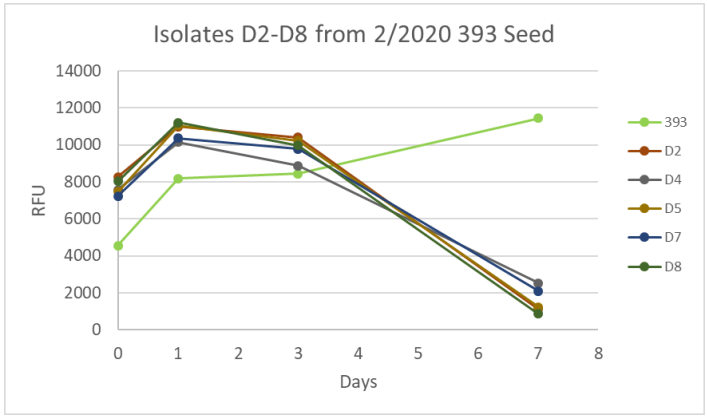
Applied Systems

- Isolated agents from pond crashes—degree of isolation may vary
- Developed for a specific field application: algal species, environmental and physiochemical parameters
- Characterization is limited to that necessary and may include
  - Basic growth characteristics –ability to replicate crashes
  - Genetic signature for detection
  - Host range
  - Temperature, salinity, major nutrients.
- Used for applied studies of detection, prevention and countermeasures.

Basic Systems

- Isolated/axenic
- Archived—cryopreservation
- Well defined host
- Growth well characterized
- Genomic sequences for host and pathogens
- Used to understand basic mechanism of host/pathogen interactions.
  - Rotifers
  - Some ciliate species
  - Some chytrid species.





393 Seed (2/2020) Plate Isolate	Top Hit	
Isolate D7	Flamella;Flamella arnhemensis	1323
Isolate E5	Flamella;Flamella arnhemensis	10891
Isolate F4	Amoeboaphelidium;Amoeboaphelidium protococcarum	714
Isolate F7	Amoeboaphelidium;Amoeboaphelidium protococcarum	1517
	Flamella;Flamella arnhemensis	1495

Strain collections contain very few pest species.

Major culture collections tend to focused on a relatively small number of species

May not reflect the true diversity pest species that are present

Many pest species do not appear in culture collections.

- Particularly obligate parasites

Published studies tend to focus on available species e.g. rotifers

DISCOVR compared a panel of algal species against most of the grazer species that could be obtained from U.S. culture collections and other commercial sources.

	Control	*Brachionus plicatilis* 10/ml	*Brachionus plicatilis* 50/ml	*Brachionus rotundaformis* 10/ml	*Brachionus rotundaformis* 50/ml	*Oxyrrhis marina* 100/ml	*Oxyrrhis marina* 1000/ml	*Euplotes* 40/ml	*Euplotes* 400/ml	*Poteriochromonas* 4K/ml	*Poteriochromonas* 40K/ml	*Bigelowiella longifila* 20K/ml	*Bigelowiella longifila* 200K/ml	*Pseudopedinella elastica* 20K/ml	*Pseudopedinella elastica* 100K/ml	*Ochromonas* 20K/ml	*Ochromonas* 90K/ml	*Goniamonas pacifica* 1500/ml	*Goniamonas pacifica* 7500/ml	*Chlorochromonas danica* 1000/ml	*Chlorochromonas danica* 100/ml	*Spumella sp.* 10K/ml	*Spumella sp.* 1K/ml	*Chrysastrella breviappendiculata* 7.5K/ml	*Chrysastrella breviappendiculata* 500/ml	*Cryptheco dinium cohnii* 5000/ml	*Cryptheco dinium cohnii* 1000/ml	*Bigelowiella longifila 242* 20K/ml	*Bigelowiella longifila 242* 4K/ml	
Salt Water																															Strain Avg
Micractinium sp. 14-F2	1	0.782	-0.071	1.018	1.124	0.971	0.629	NA	NA	NA	NA			1.049	0.961	1.010	0.728	0.971	0.932	NA	NA	NA	NA	NA	NA	0.977	0.921	0.5	0.921	0.842
Nannochloris sp. 39-A8	1	-1.360	-0.264	0.872	0.656	0.904	0.936	NA	NA	NA	NA	1.044	1.132	1.070	1.211	1.000	1.211	1.070	1.158	NA	NA	NA	NA	NA	NA	0.907	0.949	0.992	0.983	0.705
Nannochloropsis gaditana 1894	1	0.702	-0.041	0.810	0.860	0.835	0.917	NA	NA	NA	NA	0.904	0.911	0.977	0.954	1.000	0.914	1.007	1.075	NA	NA	NA	NA	NA	NA	0.907	1.087	1.052	1.105	0.834
Scenedesmus sp. 46B-D3	1	0.871	0.138	0.828	0.638	0.940	0.638	NA	NA	NA	NA	0.784	0.795	1.009	1.162	1.153	1.045	0.920	0.966	NA	NA	NA	NA	NA	NA	0.964	1.027	0.955	0.884	0.859
Nannochloropsis oceanica 1779	1	0.796	0.041	1.068	0.116	0.993	0.946	NA	NA	NA	NA			1.025	1.000	0.958	0.958	0.941	0.966	NA	NA	NA	NA	NA	NA	1.108	1.008	1.058	1.067	0.817
Pichochlorum oklahomensis	1	-0.290	0.039	0.728	0.291	1.117	1.243	NA	NA	NA	NA	0.952	0.810	1.054	1.081	0.955	1.027	1.063	1.087	NA	NA	NA	NA	NA	NA	0.902	0.971	1.069	1.019	0.783
Chlorella 4-C12	1	0.809	0.001	0.757	0.662	0.978	0.676	NA	NA	NA	NA			0.940	0.918	0.903	0.970	0.985	0.948	NA	NA	NA	NA	NA	NA	0.658	0.938	0.907	0.974	0.796
Microchloropsis salina	1	1.033	-5.400	1.049	-0.082	1.180	1.148	NA	NA	NA	NA	0.904	0.886	0.949	0.936	0.917	0.846	0.904	0.860	NA	NA	NA	NA	NA	NA	0.871	1.022	0.842	0.957	0.362
Stichococcus minor	1	0.969	-0.814	0.837	-0.109	1.054	1.124	NA	NA	NA	NA	0.914	0.938	0.875	0.984	1.016	1.023	0.945	0.922	NA	NA	NA	NA	NA	NA	0.944	0.937	0.993	0.923	0.736
Fresh Water																															
Chlorella sorokiniana 1116	1	0.923	0.765	0.755	0.558	0.645	0.571	1.016	1.016	0.980	1.106	0.964	0.924	NA	NA	NA	NA	NA	NA	1.000	1.050	1.245	1.000	1.245	0.934	NA	NA	NA	NA	0.915
Monoraphidium 26B-AM	1	1.045	1.097	1.097	1.026	0.832	0.748	0.891	0.957	1.079	1.092	0.782	0.723	NA	NA	NA	NA	NA	NA	1.112	0.939	0.894	0.933	0.955	0.944	NA	NA	NA	NA	0.953
MONOR1	1	0.791	0.755	0.791	0.827	0.718	0.218	0.957	1.034	NA	0.655	0.972	0.890	NA	NA	NA	NA	NA	NA	1.030	1.060	1.036	1.018	1.036	0.988	NA	NA	NA	NA	0.869
Acutodesmus obliquus UTEX393	1	1.160	1.180	1.220	1.280	1.150	0.480	1.068	1.102	NA	1.067	1.067	1.012	NA	NA	NA	NA	NA	NA	1.005	1.011	0.500	0.644	1.173	0.952	NA	NA	NA	NA	1.004
Chlorella sorokiniana 1044	1	1.056	0.990	0.990	1.080	0.973	0.8	1.053	1.047	0.986	0.940	0.913	0.942	NA	NA	NA	NA	NA	NA	0.854	0.988	0.988	0.878	0.915	0.915	NA	NA	NA	NA	0.962
Chlorella vulgaris LRB 1201	1	0.748	0.855	0.828	0.807	1.0256	0.938	0.872	0.862	0.878	0.857	0.722	1.044	NA	NA	NA	NA	NA	NA	0.952	1.000	1.000	0.995	0.894	1.037	NA	NA	NA	NA	0.906
Stichococcus minutus	1	0.917	1.076	1.057	1.051	0.922	1.080	0.758	0.076	NA	-0.029	0.982	1.041	NA	NA	NA	NA	NA	NA	1.023	0.983	0.989	0.955	0.949	1.000	NA	NA	NA	NA	0.872
Scenedesmus DOE 0152z	1	1.013	0.927	1.020	0.993	0.855	0.827	0.960	0.974	NA	0.918	0.966	0.986	NA	NA	NA	NA	NA	NA	1.100	1.037	0.991	1.046	1.083	1.023	NA	NA	NA	NA	0.984

Utility of applied model systems to field cultivation

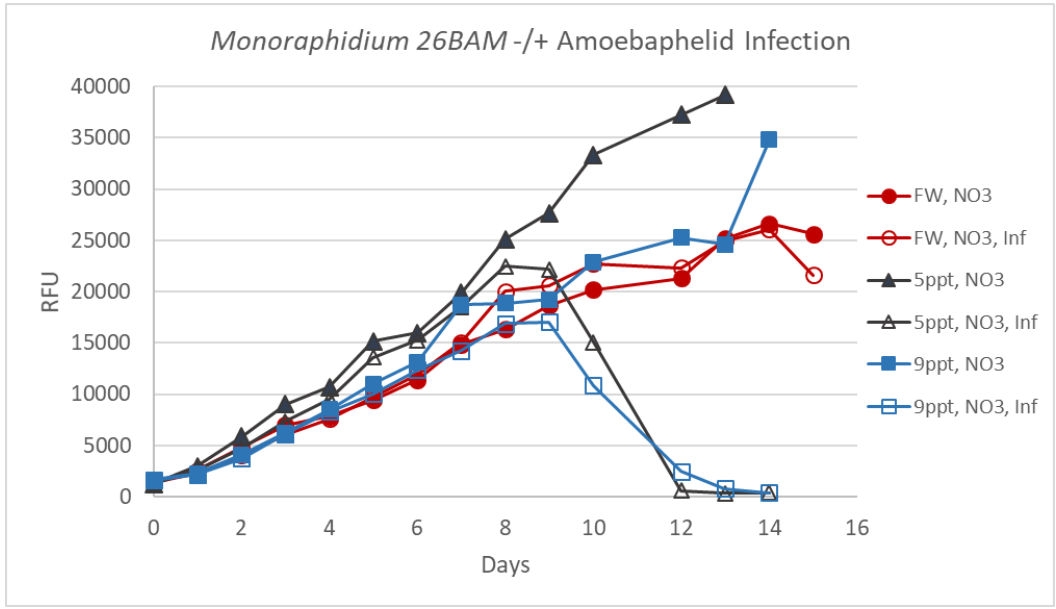
Determination of host range

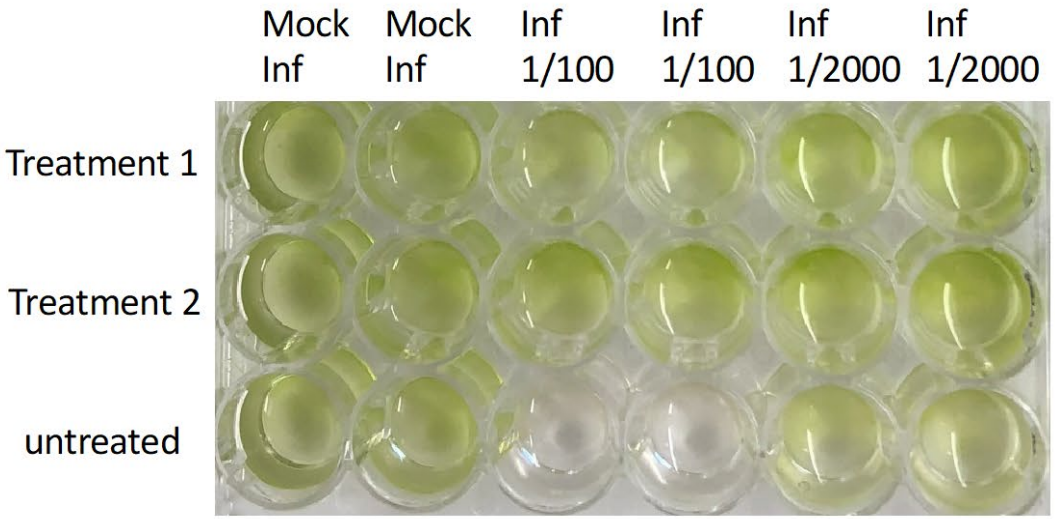
Determination of growth conditions

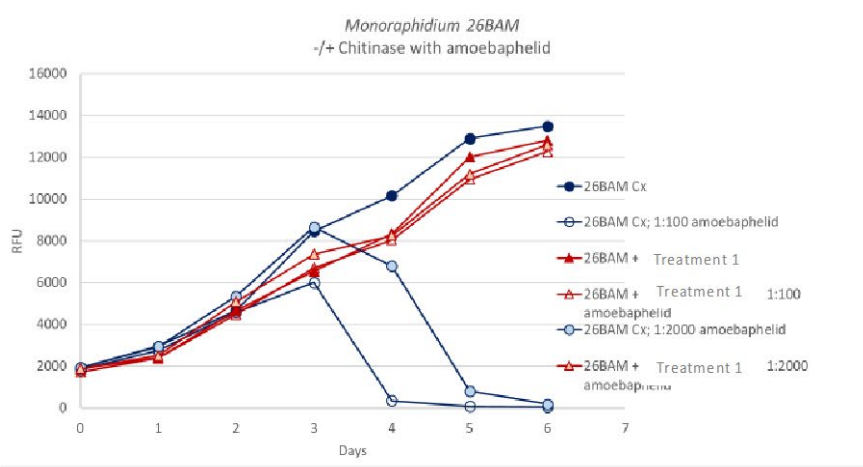
Development of early detection methods e.g PCR probes

Testing of countermeasures

- Alternative cultivation strategies
- Biocides
- Co-cultures with probiotic species
- Development of resistant phenotypes







How translatable is research in model systems?

Algal pests could be separated into 2 broad categories

General threat species, broad "host range" species that constitute threats to multiple algal species.

- Grazers
- Rotifers
- Ciliates
- Amoeba

Narrow host range threat species:

- Bacterial pathogens *e.g. Vampirovibrio chlorellavorus*
- Eukaryotic parasites, *e.g.* Chytrids, Amoeboaphelids
- Viruses

How transferable is research in countermeasures—how broad range is the countermeasure?

- Biocides e.g. fluazinam: broad antifungal activity
- Hyperparasites, viruses, and pathogens may have narrower target range

What are the best surrogate systems available for testing broad strategies?

- Research into one host/parasite system extended to field applications