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Non-Chemical crop protection treatments

Culture flow/mixing

Tetraselmis sp. algae and *Amphora* sp. diatoms were identified as potential weed strains during pest capture minipond experiments. A common distinguishing feature of both these weeds is there size relative to the crop *Nannochloropsis*. Numerous experiments with indoor and outdoor culture showed that both strains settle at a faster rate than *Nannochloropsis*, and that in healthy *Nannochloropsis* cultures stay well suspended, even during long periods without paddle wheel motivation (such as during power interruptions). This should allow separation of a mixed culture by altering mixing parameters. In addition to using this method after a weed population has established, it may also be used to successfully prevent a weed strain from establishing a significant population level within a culture.

Minipond: flow speed and schedule for weed control

Without modifying current pond structure, the only parameters available for modification are paddle wheel speed and schedule. A minipond experiment was used to test the effect of paddle wheel speed and/or schedule on an established weed population. A deliberate mixture of *Tetraselmis* sp. and *Nannochloropsis* was distributed amongst miniponds. In 4 separate phases of the experiment control ponds were set at standard paddle wheel speed and schedule (7rpm, 24hrs) and treatment ponds were set at various speeds and schedules (3rpm/24hrs, 7rpm/12hr on 12hr off, 5rpm/12hr on 12hr off). In all treatment groups the level of *Tetraselmis* weed was quantitatively reduced as compared to control ponds showing that both speed and schedule were effective weed control methods. This experiment also represented an opportunity to develop weed tracking assays using the Flowcam particle analysis equipment. A method for tracking weed species in the size range ~5-40um was successfully developed and deployed at the Columbus, NM site. Qualitatively, an effect on diatoms was also observed where lower numbers of *Amphora sp.* diatom were present in treatment groups as compared to the control (no high throughput, accurate quantification method was available at the time for assessing diatom level since this species of diatom is too large to be analyzed accurately via Flowcam and also has a tendency to stick to the equipment). Diatoms also took longer to appear in treatment group ponds highlighting the potential preventative nature of this method of crop protection. Data from the final phase of this minipond experimentation is shown in Figure 1. Based on this successful experiment similar experiments were set up using existing raceway cultures. A recommendation was made to the commercial scale site to fit timers and variable speed motors to the paddle wheels in order to render this approach available for culture maintenance.

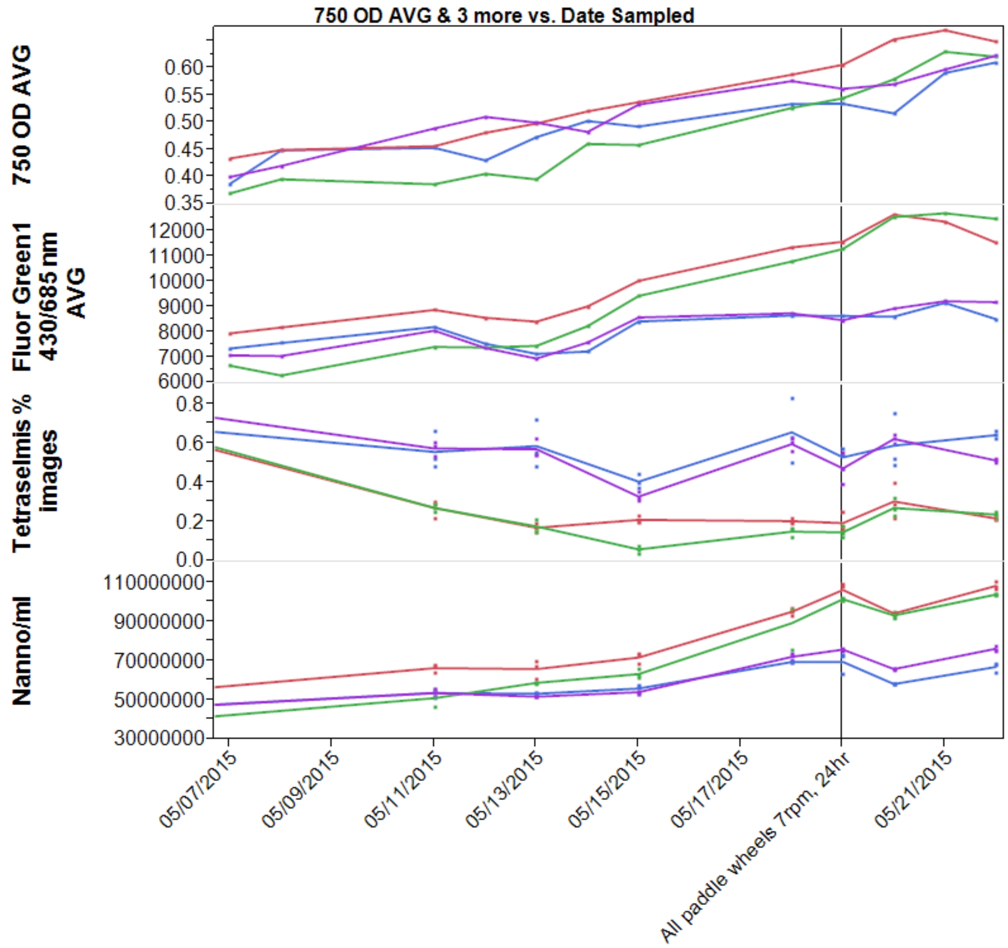

Figure 1. Experiment_0176 Paddle wheel speed/schedule. Analysis of culture composition over time. Data from four miniponds are shown. Slow speed and paddles off at night (red+green) and standard speed 24hr/day (blue+purple). OD and fluorescence measurements are from plate reader data on morning samples. % Tetramselmis images and *Nannochloropsis* concentration (Nanno/ml) is measured by Flowcam analysis of 10,000 images using a sample filtered through 50um filter. All paddle wheels were normalized to 7rpm/24hrs on 5/19.

Mechanical disruption of rotifers

Pumped ponds

In standard raceway ponds, the algal culture is circulated by the action of a single paddlewheel. An alternative method for culture circulation is the use of mechanical pumps. Pumps may offer certain advantages, such as lower energy costs and the ability to develop a selective environment that confers an advantage to the crop. One such advantage could be preventing growth of pest organisms that would either sink to the bottom of the pond due to lower flow and/or be mechanically disrupted by the action of the pump. It has been observed in pump driven ponds that the frequency of rotifers as a predator is lowered. This is presumably due to the action of the pump, killing the rotifers. Since rotifers were known to be a significant pest for *Nannochloropsis,* this hypothesis was tested with culture deliberately infected with rotifers in a pumped pond (Low Flow pond – LF) as compared to a paddle wheel pond (in this case a minipond – MP). The data for this experiment is shown below in Figure 2. Rotifers were added to the culture in the LF ponds and then part of this culture was removed for cultivation in the MPs as a control.

The rotifer population increased in the paddle wheel pond but decreased in the pumped pond suggesting that this type of pond may be protective against this type of pest.

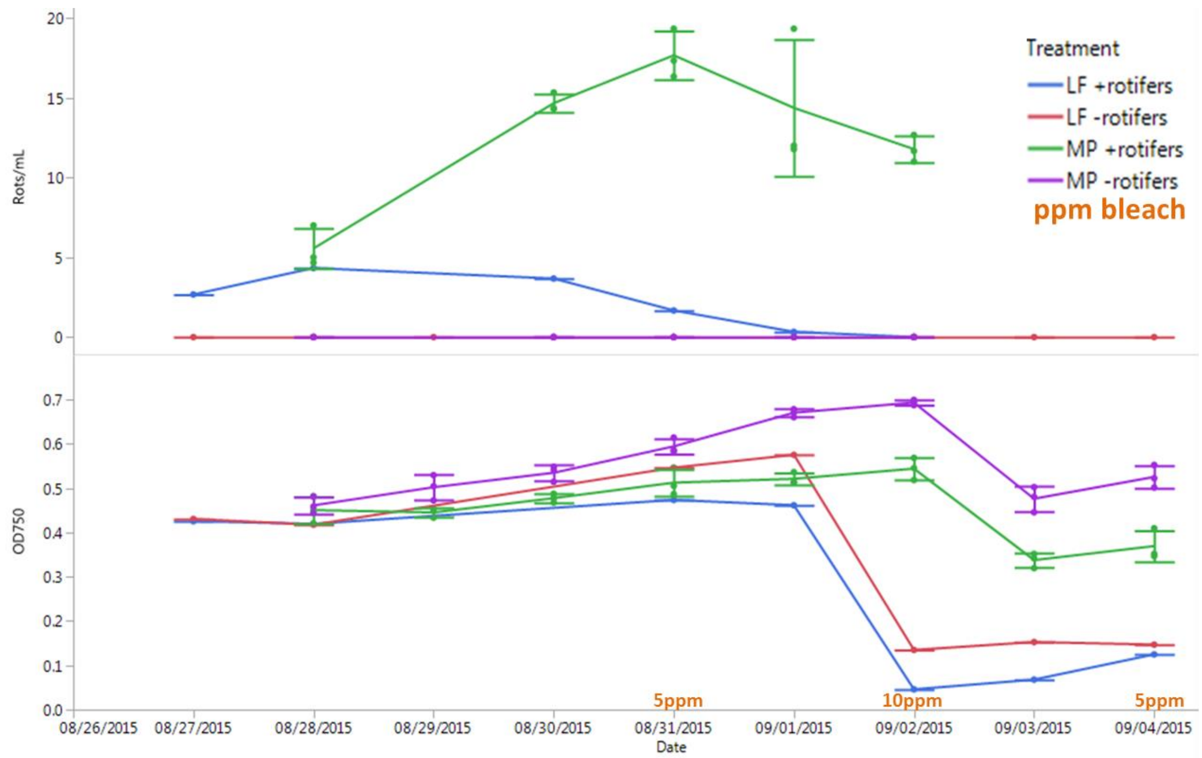

Figure 2. Rotifer and algae growth compared between different pond types. Culture with and without rotifers (+/-rotifers) was cultivated in paddle wheel ponds (MP) and pumped ponds (LF). Upper graph shows rotifers/ml in each pond type. Lower graph shows OD750nm of each pond type. LF ponds are individuals whereas MP ponds are triplicates.

External pumping for crop protection

Pumping of culture has been reported to successfully prevent propagation of certain pests, especially rotifers. Observations in ponds where culture is moved by pump also suggest that pumping may be a viable crop protection method (see above). Attempts at various scales (from lab to production pond) to show an effect of pumping via an external pump (i.e. not part of the cultivation system) on rotifer population did not show any effect on rotifer population. This suggests that positive effects observed elsewhere are likely due to higher forces in those systems, or more likely due to the increased frequency that a rotifer would be in contact with pump forces. Pumping the entire culture volume at a high frequency is not scalable since the pump required at a 1+ acre sized pond would become too large to be practical or economical. More work should be done to determine the minimum required shear force and contact frequency to effectively control grazer pests such as rotifers as it is possible that we just haven't yet identified the optimum conditions of commercial scale implementation.

High throughput testing for weed control

High throughput screening methods available at the San Diego site were utilized to assess the impact of environmental conditions on historical and newly identified weed strains. Weed strains were chosen based on their presence in cultivation ponds this year and previous laboratory experiments that had shown they were able to grow in current cultivation media. Two of the strains were also observed in proactive weed capture experiments described in section xxx (SE61336 - Chlorella sp. and SE61268).

Strain	Mean								
SE61280	0.01057				D	E			
SE50022	0.00941					E	F		16°C
SE61272	0.00930					E	F		
SE50025	0.00929					E	F		
SE61336	0.00595							G	
SE61268	0.00507							G	
SE61336	0.01814	A	B						
SE61280	0.01676		B						25°C
SE50025	0.01604		B	C					
SE50022	0.01581		B	C					
SE61272	0.01346			C	D				
SE61268	0.00974					E	F		
SE61336	0.02067	A							
SE61280	0.01604		B	C					
SE50022	0.01598		B	C					32/25°C
SE50025	0.01527		B	C					
SE61272	0.01338			C	D				
SE61268	0.00730						F	G	

Figure 3. Statistical analysis of growth rate of *Nannochloropsis* compared to weed strains at different temperature profiles. Strain name, growth rate (OD750nm change/day) and connected letter report are shown. Strains were grown in the microplate growth rate assay (MGRA) at three different temperatures (show on right). Temperature profile 32/25°C used 32°C when lights were on and 25°C when lights were off. Groups connected by the same letter cannot be statistically distinguished (Tukey-Kramer, p=0.05). The *Nannochloropsis* strains are highlighted in green.

This experiment showed that in all conditions tested at least one weed strain was able to grow at a similar or better rate than the crop, highlighting the need for methods to prevent contamination and propagation of such organisms in the ponds. As mentioned in other sections of this report, weeds of the genus *Chlorella* such as SE61336 are of particular concern since they are not easily distinguished from *Nannochloropsis* by methods currently in use for pond monitoring.

In previous years it was observed that the method of harvest was more successful at removing crop from the culture than the weeds and thus this step in the cultivation process acted as a selection for the predominance of weed strains. Now that weed strains have been identified for the current crop and media conditions it is essential to test all parts of the cultivation process at production scale to identify any potential actions that may result in selection against the crop and for the weeds (or indeed any other pest organism). This research may also identify opportunities to apply an advantageous selective pressure as part of cultivation practices i.e. one that selects for the crop and against pests.

High throughput methods such as the one described above are powerful tools for assessing the effect of conditions on pest pressure. Information from such experiments this year enabled the choice of which weed species to focus crop protection efforts on, as well as giving information on when to expect certain weeds to potentially appear in the culture. Future applications of this method could include chemical treatments or media manipulation to investigate the possibility of modifying the culture environment to favor propagation of crop over weed strains. This is another example where the high throughput technologies available in SEI laboratories can quickly and directly affect research focus at larger scale.