Stress Rx™ Research Results

Stress Rarely Travels Alone.

Drought and heat are often companions; salinity stress mimics drought stress; UV damage and heat go hand in hand; and almost any stress makes plants more susceptible to insects and diseases.

Considering this, we began screening a variety of ingredients for their ability to increase turf's stress tolerance. Our goal was to harness the stress-fighting capabilities of seaweed extract (SWE) and add other natural active ingredients that would synergistically improve stress tolerance.

This paper shows the research results from Rutgers and Virginia Tech over several years. The ingredients that performed best under multiple and simultaneous stresses of heat, drought, high UV, and salt became **Stress Rx™.**

Drought and Summer Stress
Rutgers University
Year 1, 2010

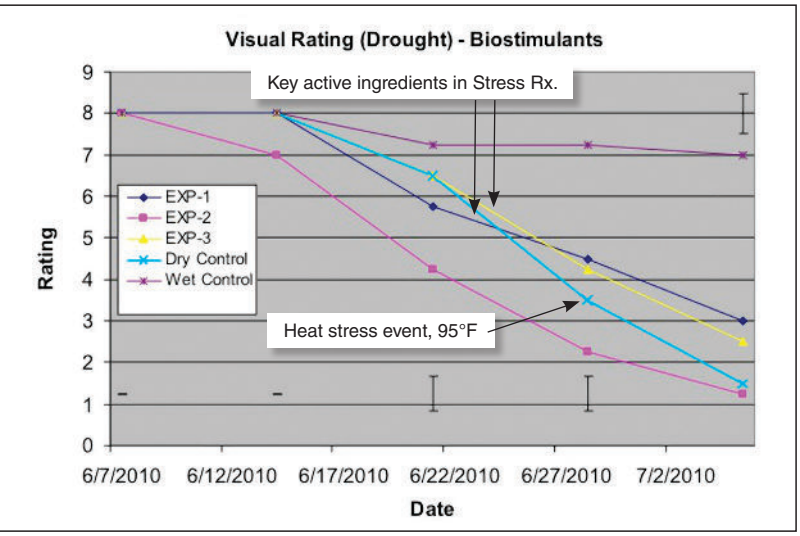
Study Objective: Seaweed extract from *Ascophyllum nodosum*, *Laminaria* and other North Atlantic seaplants naturally contains glycinebetaine, a known osmotic protectant (a molecule that protects cells from dehydration under salt and drought stress). The aim of this study was to evaluate whether Ocean Organics (OO) seaweed extract (SWE) enhanced with additional glycinebetaine (from another proprietary natural material) would improve drought tolerance of turf.

Study Conditions (Field Study Year 1): Individual plots of Creeping Bentgrass cv. '007' were maintained at 0.375" mowing height. Pre-drought applications of Ocean Organics seaweed extract, enhanced with additional naturally-derived glycinebetaine (EXP 1), occurred twice prior to water withholding. Field plots had only 2.23" rain throughout drought period. Applications of stress protectants occurred three times during the drought period, followed by rewatering of site. Post-drought applications of stress protectants occurred three times.

Results Year 1: (Bingru Huang and Patrick Burgess, Rutgers University, *Evaluation of Ocean Organics' Biostimulant Stress Protectants for Improving Drought Tolerance and Post-drought Recovery of Creeping Bentgrass*, 2010).

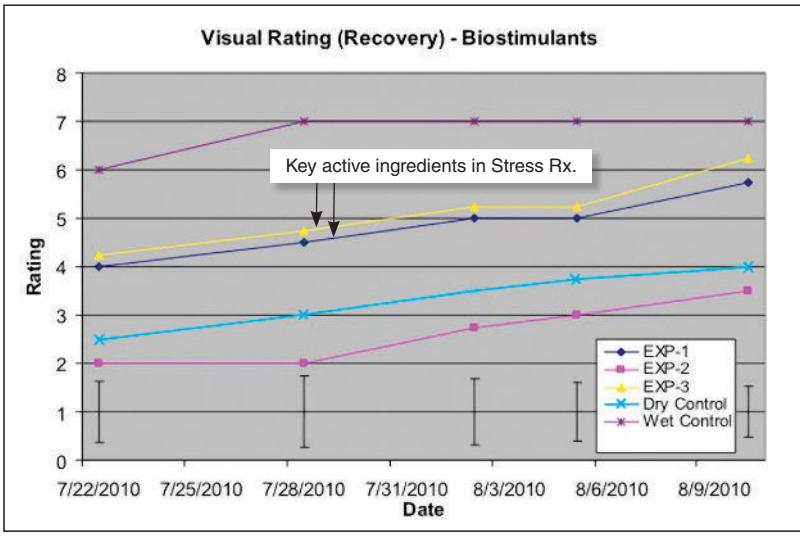
- "With the onset of major heat stress on 28 June, [OO SWE + Glycinebetaine] maintained a significantly higher Total Quality than the unwatered control."
- "Plots treated with [OO SWE + Glycinebetaine] displayed significantly higher Relative Water Content than the unwatered control and maintained RWC close to that of the watered control."
- "Based on the data collected during the experimental period, plots treated with [OO SWE + Glycinebetaine] resulted in significantly better Turf Quality, not only from a visual standpoint, but from a physiological perspective as well."
- "After two weeks of regular watering, plots treated with [OO SWE + Glycinebetaine] maintained significantly higher TQ [total quality] than the unwatered control…"

Active ingredients in Stress Rx improved quality under heat and drought stress and also during drought recovery.



Visual Rating of Turf Quality (TQ) *during the water withholding period* (4 June – 5 July) averaged among chemical treatments and controls (P<0.05).

Active ingredients in Stress Rx show improved quality under heat and drought stress.



Visual Rating of Turf Quality (TQ) *after two-weeks of rewatering* (22 July – 11 August) averaged among chemical treatments and controls (P<0.05).

Active ingredients in Stress Rx [OO Seaweed + Glycinebetaine] show improved quality during recovery.

Drought and Summer Stress
Rutgers University
Year 2, 2011

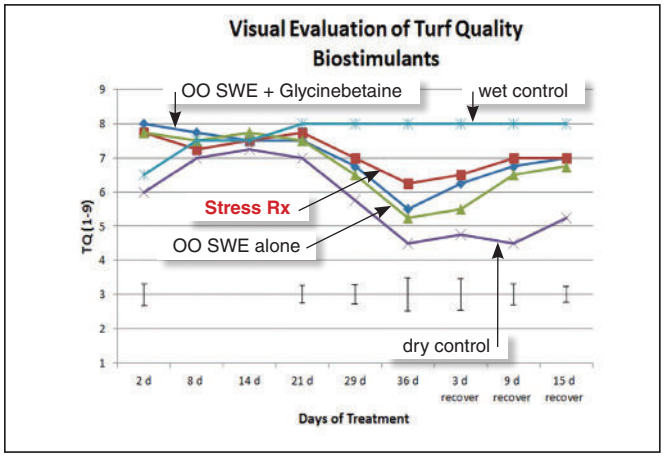
In addition to again testing Ocean Organics seaweed extract and Glycinebetaine (which had good results in year 1), we tested an improved formulation which eventually became "Stress Rx."

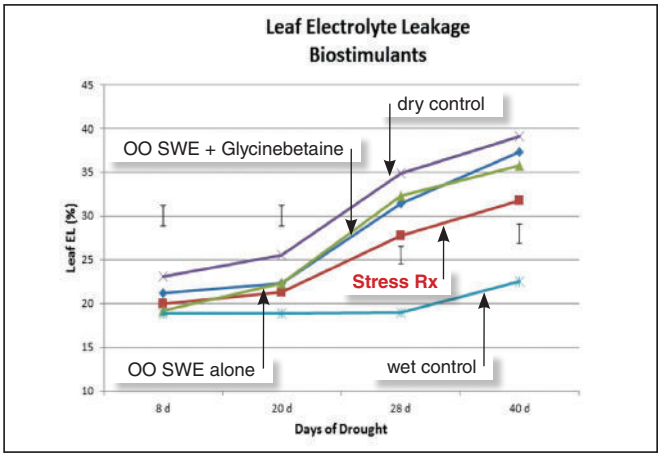
Study Conditions (Field Study Year 2): Individual plots of Creeping Bentgrass cv. '007' were maintained at 1.0 cm mowing height. Pre-drought foliar applications of experimental stress protectants occurred twice. Drought period was initiated by terminating overhead irrigation and ended July 18 with site rewatering. Net rainfall during the 40-day drought period totaled 11.53 cm and experimental stress protectants were applied five times, occurring weekly. Post-drought recovery began on July 19 by rewatering the site to field capacity. Recovery lasted 15 days, during which time stress protectants were applied three times.

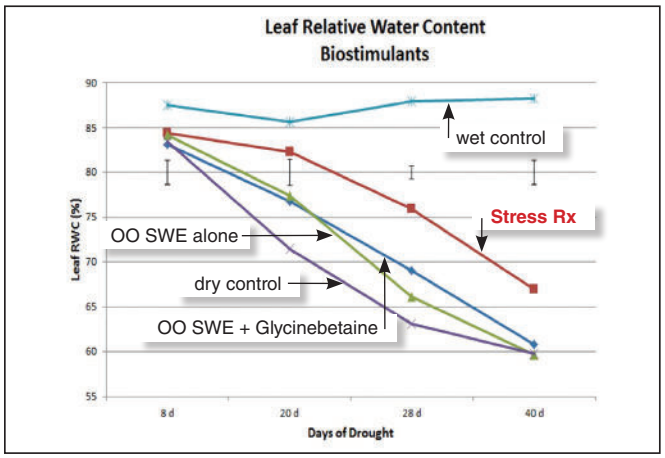
Results: (Patrick Burgess and Bingru Huang, Rutgers University, *Evaluation of Ocean Organics' Biostimulant Stress Protectants for Improving Drought Tolerance and Post-drought Recovery of Creeping Bentgrass*, 2011).

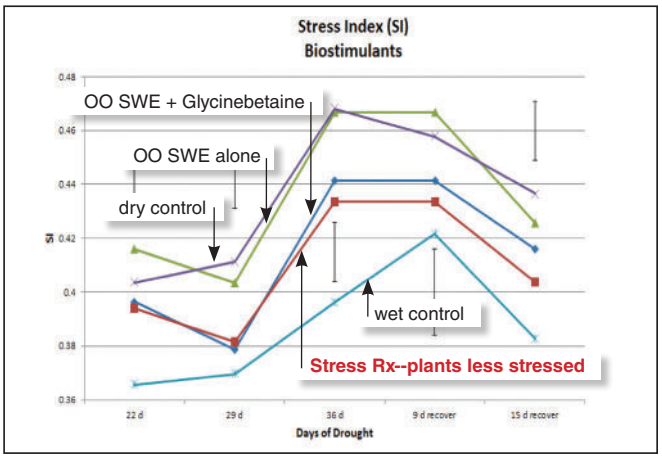
- "Notable differences between treatments became statistically significant after 21 d water withholding, at which time SWC [soil water content] dropped below 20% indicating that plots were exposed to substantial drought stress. At this time, all plots treated with any of the three [OO] experimental products had significantly better TQ [total quality] than the unwatered checks. This improved quality was due to higher leaf RWC [relative water content], less membrane damage via lower EL [electrolyte leakage], and better osmotic adjustment."
- "Towards the end of the drought period, as SWC approached 15% and water deficit became severe, plots treated with experimental [Stress Rx] continued to display the best visual quality of all non-irrigated plots. Following collections of leaf samples, it was discovered that applying [Stress Rx] promoted far better RWC, EL, and OP [osmotic potential] than both the unwatered checks and those plots treated [with OO SWE + Glycinebetaine] or seaweed alone."

With intense heat events during the trial, Stress Rx kept quality ratings between 6-8 throughout the drought and recovery stages, helping maintain better relative water content, membrane integrity and osmotic adjustment than controls.









Plots treated with Stress Rx had higher quality ratings (top left) and higher relative water content (bottom left) compared to the dry controls. Stress Rx provided additional statistical benefits over [OO Seaweed + Glycinebetaine] or Seaweed alone.

Plots treated with Stress Rx had less membrane leakage (top right) and a lower stress index (bottom right). In both cases, lower numbers are better. During the trial, temperatures hovered around 30°C (86°F), reached 95°F nine times, and even exceeded 104°F.

Note: [OO SWE + Glycinebetaine] improved quality in both 2010 and 2011. The final Stress Rx formulation, which includes additional ingredients, was finalized and tested only in 2011.

Heat and UV Stress
Virginia Tech, 2012

Study Objective: Heat and UV-B stress can create a lethal combination during tough summer conditions.

Our goal is to provide products that boost stress tolerance and give superintendents an added level of protection.

In particular in these trials, we wanted to know if Stress Rx could lengthen roots during stress and recovery. Growth chamber studies make it easier to study root length and development. Erik Ervin and his post-doc Xunzhong Zhang created a novel trial to test both heat and UV-B stress in heat chambers.

Study Conditions: We screened various natural compounds in combination with seaweed extract for improving creeping bentgrass tolerance to heat and UV-B stress. Mature 'A4' creeping bentgrass plugs (5 cm diam.) were taken from field plots and transplanted into containers in mid-October. The turf was grown under a mist system in a greenhouse. All substances were applied at 6 oz/1000 ft^2 (diluted to 1 gal). Before 1st application, roots were trimmed to 1 cm; shoots were trimmed to 1 inch height. After two applications, the grasses were placed in a tank with half Hoagland's solution and subjected to UV-B and heat (95/80°F) stress in a growth chamber.

The photosynthetic photon flux was 400 $\mu mol/m^2/s$. The treatments were applied at day 3 and day 8 after UV+heat stress initiation. The UV-B and heat stress caused complete leaf firing [necrosis] by day 12 in Trial 1, so in Trial 2, the temperature was reduced to 90/77°F (day/night) and the duration of stress was shortened to 8 days.

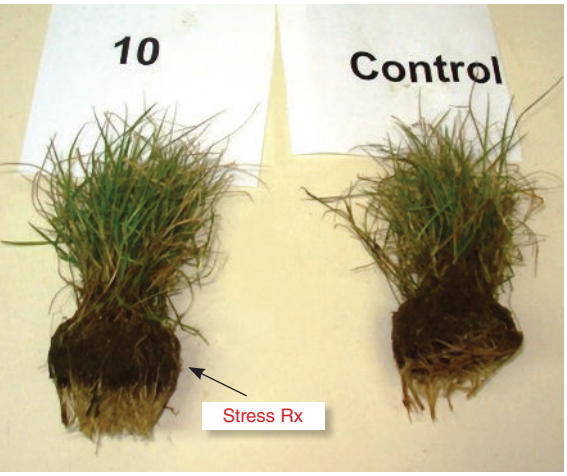
Results: Treatment with Stress Rx statistically improved root length *in both Trial 1 and Trial 2* during the heat/UV-B stress period. With Stress Rx treatment, roots were between 35 to 87% longer than in controls without Stress Rx. In Trial 2, recovery measurements were also taken. Stress Rx treatments had 54% longer roots than controls after 8 days of recovery.

During the UV/heat stress and the recovery phase, Stress Rx and other Ocean Organics seaweed combinations improved quality and had roots that were longer and healthier than controls.

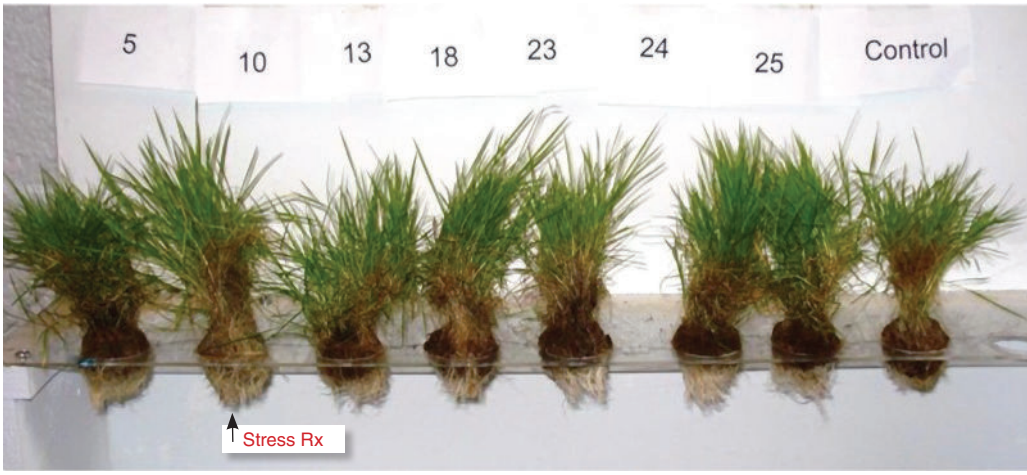
	Leaf Firing Control	Leaf Firing Stress Rx	Root Length (cm) Control	Root Length (cm) Stress Rx (% longer than control)
Trial 1				
6 Days Post-onset of UVB/Heat Stress	7a	5.5b-e	2.3d	4.3a-c (87%)
12 Days Post- onset of UVB/Heat Stress Stress	9a	9a	2.0e	3.5ab (75%)
Trial 2				
4 Days Post-onset of UVB/Heat Stress	5.8a	4.8de	2.0e	2.7a-d (35%)
8 Days Post-onset of UVB/Heat Stress; Recovery Begins	6.6a	5.5c-f	2.3d	3.5a (52%)
Day 16 after 8 days of recovery	3.3a	2.5bcd	2.4f	3.7ab (54%)

Mean followed with same letters in each column are not significantly different at P ≤0.05.

Stress Rx increased root length and improved quality. Leaf Firing is measured on a scale from 1-9, with 9 being complete firing; less firing is better, indicating better quality.



On day 8 of heat and UV-B stress during Trial 2 at Virginia Tech, turf treated with Stress Rx had roots that were on average 52% longer than the controls.



Even on day 36 of Trial 2 at Virginia Tech (28 days after the end of the stress period), turf treated with Stress Rx and other Ocean Organics seaweed combinations had roots that were longer and healthier than the controls.

Salt Stress
Rutgers University, 2008

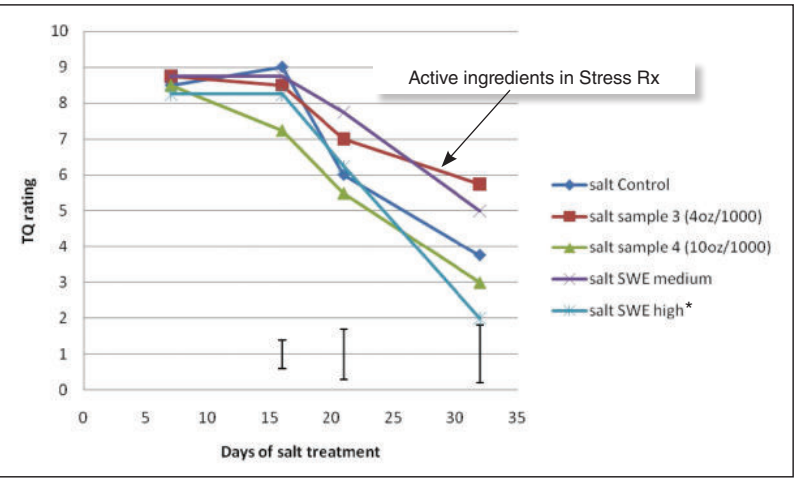
Study Objective: Seaweed extract from *Ascophyllum nodosum* naturally contains glycinebetaine, a known osmotic protectant (a molecule that protects cells from dehydration under salt and drought stress). The aim of this study was to evaluate whether Ocean Organics seaweed extract enhanced with additional glycinebetaine (from a proprietary natural material) would improve salinity tolerance of turf. These became the active ingredients in Stress Rx.

Study Conditions: Creeping bentgrass 'Penncross' plugs were established and maintained under greenhouse conditions. Plants were then sprayed with Ocean Organics seaweed extract, enhanced with additional naturally-derived glycinebetaine [OO Seaweed + Glycinebetaine] at 4 oz/1000 sq feet for two weeks prior to salinity stress treatment consisting of a 12 ds/m salt solution. During salinity treatment, plants were sprayed weekly with [OO Seaweed + Glycinebetaine] at the given rate.

Results: (Bingru Huang and Emily Merowitz, et al., Rutgers University, *Physiological Effects of Biostimulants on Turfgrass Salinity Tolerance*, 2008)

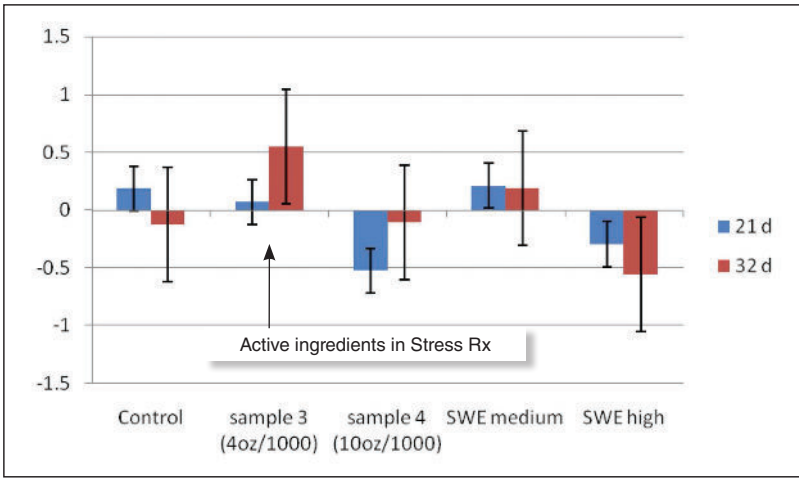
- "Plants that were treated with [OO Seaweed + Glycinebetaine] at 4oz./1000 sq ft were able to maintain higher Total Quality levels throughout the duration of salt treatment compared to control plants sprayed with water only."
- "Relative Water Content of the salt-stressed plants sprayed with [OO Seaweed + Glycinebetaine] was maintained at a significantly higher level."
- "Osmotic Adjustment levels were highest for plants treated with [OO Seaweed + Glycinebetaine].
- "Our results suggest that application of [OO SWE + Glycinebetaine] served to increase creeping bentgrass tolerance of salt stress most likely by maintaining better cellular hydration and water relations through accumulating glycine-betaine and increasing osmotic adjustment."

Treated plants maintained higher Relative Water Content, better Cellular Hydration and better Water Relations throughout salt treatment.



Turf Quality ratings (1 = dead, 9 = healthy) of salt stressed plants.
Active ingredients in Stress Rx improved Turf Quality ratings.

*Note: "SWE High" was an experiment to see if we could get glycinebetaine levels higher using an extremely high concentration of solids in the seaweed extract (higher than would ever be used in normal applicatons). As you can see, this was not successful; hence why we instead enhance our SWE with other proprietary natural sources of glycinebetaine to increase glycinebetaine levels.



Osmotic Adjustment calculated as the difference between sufficiently watered and salt stressed plants exposed to the same spray treatment.
Higher osmotic adjustment numbers are better.
Stress Rx active ingredients improved Osmotic Adjustment of salt stressed plants.

*Note: Refers to charts on pages 1, 2 and 4: LSD bars indicate significance among treatments.

Ocean Organics®

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