OHP: 2019 Kalmor /Nostoc Control Project Final Report

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Background:

Nostoc sp. or cyanobacteria or blue-green algae is becoming a major issue in US nurseries. Cyanobacteria is very important due to its classical activities like nitrogen fixation (Rizvi Rimsha et al., 2014). *Nostoc* can take N_2 (nitrogen gas) from the air and use it in symbiotic relationships with mosses (Fig. 1), ferns, lichens and other fungi. *Nostoc* belongs to the family Nostocaceae in the cyanobacterium (Rizvi Rimsha et al., 2014). *Nostoc* pre-dates the appearance of vascular plants on earth by about 700 million years, making it one the most primitive organisms known to man. Some scientists believe cynaobacteria produced the first oxygen for Earth's early atmosphere. *Nostoc* has no roots, stems or leaves, no vascular tissue, no flowers, seed or fruit, it does not even develop an embryo from the zygote (fertized egg). It is, however, a plant but in the sub-group thallophyta, which pre-dates even the bryophytes (liverworts and mosses) as cyanobacteria lack any well-differentiated body structures. Its only commonality with plants is that they contain chlorophyll and therefore can make their own food (Shuttleworth and Zim, 1967).

Fig. 1. One of the predominate functions of *Nostoc* is nitrogen fixation creating symbiotic relationships as shown here with moss (Photo by: H. Mathers).

Cyanobacteria have high adaptive potential and can occur in the most extreme habitats, including hypereutrophic lakes or water bodies that are characterized by excessive nutrient concentrations [e.g. nitrogen (N) and phosphorous(P)] with algal blooms and periods of oxygen deficiency (Dvornyk and Nevo, 2004). *Nostoc* can exist in hot springs, heavily polluted water or in moist field tiles (Shuttleworth and Zim, 1967). These adaptive capabilities of cyanobacteria are related to their ability to “morph” or take on different forms when under stress (Dvornyk and Nevo, 2004). This is termed polymorphism. These forms may be called alternative phenotypes. One phenotype occurs when *Nostoc* is dry. When dry it maybe barely noticeable, forming dark-brown to black, flaky, paper-like sheets (Fig. 2). When wet, it swells up and forms conspicuous green to greenish-brown globby, squishy mats (Fig. 3). These mats can carpet alleyways and container nursery yards, especially if the ground is compacted and poorly drained (Parke and Stoven, 2014). The colonies are covered by a jelly-like sheath that contributes to the colony's ability to tolerate extended periods of drought and freezing temperatures (Fig. 3). These characteristics have also made *Nostoc* challenging to control in nursery and landscape settings, where it has become widespread on soil, gravel (Fig. 2), ground cloth (Fig. 3) and in turf.

Fig. 2. (Left) Two polymorphisms or phenotypes of *Nostoc* are shown in a MI container nursery yard. On the left of the photo is the brown flaky, paper-like sheets of one phenotype. On the right we see the black, globby mat like phenotype adjacent to the brown phenotype. (Photo by: H. Mathers).

Fig. 3. (Left) When wet, *Nostoc* swells up and forms conspicuous green to greenish-brown globby, squishy mats as another phenotypic form. The jelly-like sheath that protects the *Nostoc* is visible towards the bottom of the photo. (Photo by: H. Mathers).

The ideal environment for *Nostoc* is in pH range of 7.0 to 8.5, with a lower pH limit of 5.7 (Allison et al., 1937). Therefore, acids are more effective than bases for control. Especially fatty acids, such as pelagonic acid or Scythe, since cyanobacteria are felt to be the basis of fatty acid development on earth – and "likes act against likes." However, no herbicide aicd or fatty acid has proven commercially acceptable in control. *Nostoc* grows best when light intensity is less than that of direct sunlight, but can continue to grow and fix nitrogen in the presence of glucose and absence of sunlight (Allison et al., 1937). It is considered that 20% of all known cyanobacteria occur in saline conditions and a majority of them being truly marine.

Finding a control is critical, as it is not just an unsightly nuisance but a slip-hazard in the workplace. Like all thallophytes, *Nostoc'*s have single-celled reproductive structures and reproduce asexually by cell-division and by spores that can be motile or non-motile (Shuttleworth and Zim, 1967). It is important to note that because they easily propagate asexually – *Nostoc* does spread by shoes, on pots, equipment and grading of gravel. If you attempt to grade the gravel container yard to eliminate *Nostoc*, you will actually spread the *Nostoc* throughout the nursery.

Objective:

The study that is the subject of this report, was designed to evaluate Kalmor® (EPA Reg. No. 91411-TX-001) (Copper hydroxice* 46.1%) (*Metallic copper equivalent 30%) (OHP, Inc., Bluffton, SC) for potential control or suppression of *Nostoc* sp. on nursery gravel pads. Kalmor® is an OMRI registered Fungicide/Bactericide and maybe used in greenhouses and shadehouses. The current label provides rates for application to specified ornamental crops of 0.5 to 2.0 lbs/ac with 100 gal/ac application volumes. However, the rates used in this study are much higher as we were evaluating on non-crop areas and the surfaces on which nursery container crops are grown. The same application volume, however, of 100 gal/ac was used.

Material and Methods:

The study design was a randomized complete block design (RCBD) with three replications on gravel container yards with *Nostoc* sp. pressure. The trial was initiated on June 24, 2019 at Acorn Farms, Inc. (Wholesale Nursery), Galena, OH. It was bright and sunny and 90°F at the time of initiation. The area where the trial was located received excessive irrigation and had algae, *Nostoc*, moss and nutsedge growing on the site. Standing water was not-uncommon. The recommended test areas of 50 ft^2 split in half to provide one application to 25 ft^2 on ½, and two applications on the other ½ or 25 ft^2, could not always be located with three replications per treatment. Therefore, variuos sized plots of minimum 24 ft^2 were used throughout but not always adjacent to its 2 application counterpart, ex. treatment 1 and 4 were counterparts. Treatment #1 received 1 application, and treatment #4 received 2 applications, of the same rate of Kalmor (Table 1). Likewise, treatments 2 and 5, and treatments 3 and 6, were also considered counterparts. Plot sizes according to replication and treatment are provided in Table 1. For treatments 4, 5 and 6 that required two applications the second application was made four weeks after the first or (4WAT). A CO_2 backpack sprayer delivering 50 gal/ac (R&D Sprayers, Opelousas, LA 70570) using a single wand attachment with one 8005VS nozzle (TeeJet, Carol Stream, IL 60116), applying a 24" band was used. Sprays were conducted in two directions, i.e, up and back. These twice over applications ensured a superior coverage vs once over and 100 gal/ac delivery. Evalutions were conducted at 30 days after treatment (DAT), 60 DAT and 90 DAT (Fig. 5A) for treatments receiving one application. For treatment numbers #4, 5 and 6, evaluations were conducted at 30 DAT, 30 DA2T and 60 DA2T (Table 1) (Fig. 5B). Four dates of evaluation are presented (Table 1 and Fig. 5 A and B). The date of initiation was included as not all plots started with the same *Nostoc* pressure (labelled as percent control) (Table 1 and Fig. 5 A and B). The main effects of treatment (Fig. 4) does not include the initiation pressures of *Nostoc*.

Results:

The interaction effect of treatment by date was significant; therefore, main effects would generally not be presented. However, the main effect of treatment is added to better illustrate the differences between one application and two with the same rate of Kalmor (Fig. 4). For ease of viewing, the six treatments were separated into two graphs, according to number of applications (once Fig. 5A, or twice Fig. 5B) or counterpart treatments (as explained above). The control (treatment 7) was not graphed as no change was found in *Nostoc* control without an application of Kalmor (Table 1) (Fig. 12 A, B and C). Kalmor applied at 10 lb/ac once or twice provided higher levels of control, although these levels were not significantly higher than the 5 lb/ac applied once or twice by the third and fourth date of evaluation (Fig. 5 A and B). We concluded there was no statistically significant benefit to applying the 10lb/ac, 5 lb/ac or 2.5 lb/ac rate as they all reached the same level of control by the conclusion of the trial (Fig. 5 A and B). One application was sufficient to achieve 90% control or better at 90 DAT when pooled over replication. The 2.5 lb/ac rate was significantly different than the 10 lb/ac rate at 30 and 60 DAT with one application (Fig. 5 A), and two applications at 30 DA2T. By 60 DA2T or 90 DAT there were no significant differences between the three Kalmor rates of application with one or two applications (Fig. 5 A and B). Although rate was not significant statistically, horticulturally the 10 lb/ac rate was significantly different in the time to obtain control. In a nursery where containers are being moved from site to site and *Nostoc* is transferred with the pots, achieving faster control is important.

There seemed to be no statistical significance to initial *Nostoc* pressure on later control. The initial *Nostoc* pressure was significantly less in the 10 lb/ac twice applied plots versus the once applied plots. Nonetheless, the once applied and twice applied reached the same control levels at 30 DAT and 60 DAT. The initial Nostoc pressure in the 5 lb/ac Kalmor plots were not significantly different between once applied and twice applied. However, the pressure at initiation, with the 2.5 lb/ac rate, with two applications was significantly higher than in the once applied. Again, starting pressure seemed to have no impact on later control as the 2.5 lb/ac rates applied once or twice were not statistically different by 30 DA2T (Fig. 5B) or 60 DAT (Fig. 5A).

Location did seem to be important (Fig. 6) as the replication by treatment interaction was significant. Replication three was less efficacious than replications one and two, in all but one occasion (Fig. 6). The exception was treatment two, in replication two, where the *Nostoc* control was less than in replication three (Fig. 6). All three replications were placed in extremely wet areas as originally indicated. It was not uncommon to have standing water in replication one and two (Fig. 8). Replication three, moreover, was positioned in an area where water flowed through at least 25-50% on the right side of the plots, bringing with it a constant stream of potential re-inoculation from *Nostoc* upstream, sediments and algae were contained in the flow (Fig. 7). Treatment 4 in replication 3, was one of the least efficacious treatments in the study at only 75% control on 09/30/2019 (Fig. 13 C) (Fig. 6). We speculated that the resurgence of *Nostoc* by the trial completion, in this replication, was due to the flow of drainage water through replication three, with its highest flow in the treatment four plot. This decline in control, in this replication, caused the slight drop in control found with the 10 lb/ac rate pooled over replications (Fig. 5 B) at 60 DA2T.

Conclusions:

There were no significant differences in control between the three rates when pooled over replication. However, in terms of quick control 5 and 10 lb/ac rates were superior. We recommend Kalmor for *Nostoc* control, at any of the rates tested. All rates in all replications provided 70% or better efficacy, which is generally considered commercially acceptable weed control.

Table 1. The raw data from the *Nostoc* sp. control trial with Kalmor® (EPA Reg. No. 91411-TX-001) (Copper hydroxice* 46.1%) (*Metallic copper equivalent 30%) (OHP, Inc., Bluffton, SC) initiated on June 24, 2019 at Acorn Farms, Inc. (Wholesale Nursery), Galena, OH. Percent control was collected on three dates 07/26/2019, 08/22/2019 and 09/30/2019. The *Nostoc* control at trial ititaion was also measured as all plots varied in their level of *Nostoc* infection at the start.

Tmt # - Rep #	**Product**	**Rate (lb/ac)**	**Application(s)**	**Area (sq/ft)**	**Initial *Nostoc* Pressure (% control) (06/24/19)**	**30 DAT (% control) (07/26/19)**	**60 DAT and 30 DA2T (% control) (08/22/19)**	**90 DAT and 60 DA2T (% conrol) (09/30/19)**
1-1	Kalmor	10	1	25	20	100	100	100
2-1	Kalmor	5	1	24	10	100	100	100
3-1	Kalmor	2.5	1	24	20	80	80	100
4-1	Kalmor	10	2	25	5	100	100	100
5-1	Kalmor	5	2	24	40	80	100	100
6-1	Kalmor	2.5	2	25	70	80	80	100
7-1	Kalmor	0	No	10	40	0	0	0
1-2	Kalmor	10	1	24	10	90	95	98
2-2	Kalmor	5	1	24	30	70	70	95
3-2	Kalmor	2.5	1	36	60	90	90	95
4-2	Kalmor	10	2	72	80	90	95	100
5-2	Kalmor	5	2	72	40	90	100	98
6-2	Kalmor	2.5	2	30	60	90	95	98
7-2	Kalmor	0	No	24	40	0	0	0
1-3	Kalmor	10	1	30	20	95	95	90
2-3	Kalmor	5	1	30	40	85	95	75
3-3	Kalmor	2.5	1	24	40	80	80	92
4-3	Kalmor	10	2	30	10	95	95	75
5-3	Kalmor	5	2	30	10	80	90	98
6-3	Kalmor	2.5	2	25	70	80	80	80
7-3	Kalmor	0	No	21	10	0	0	0

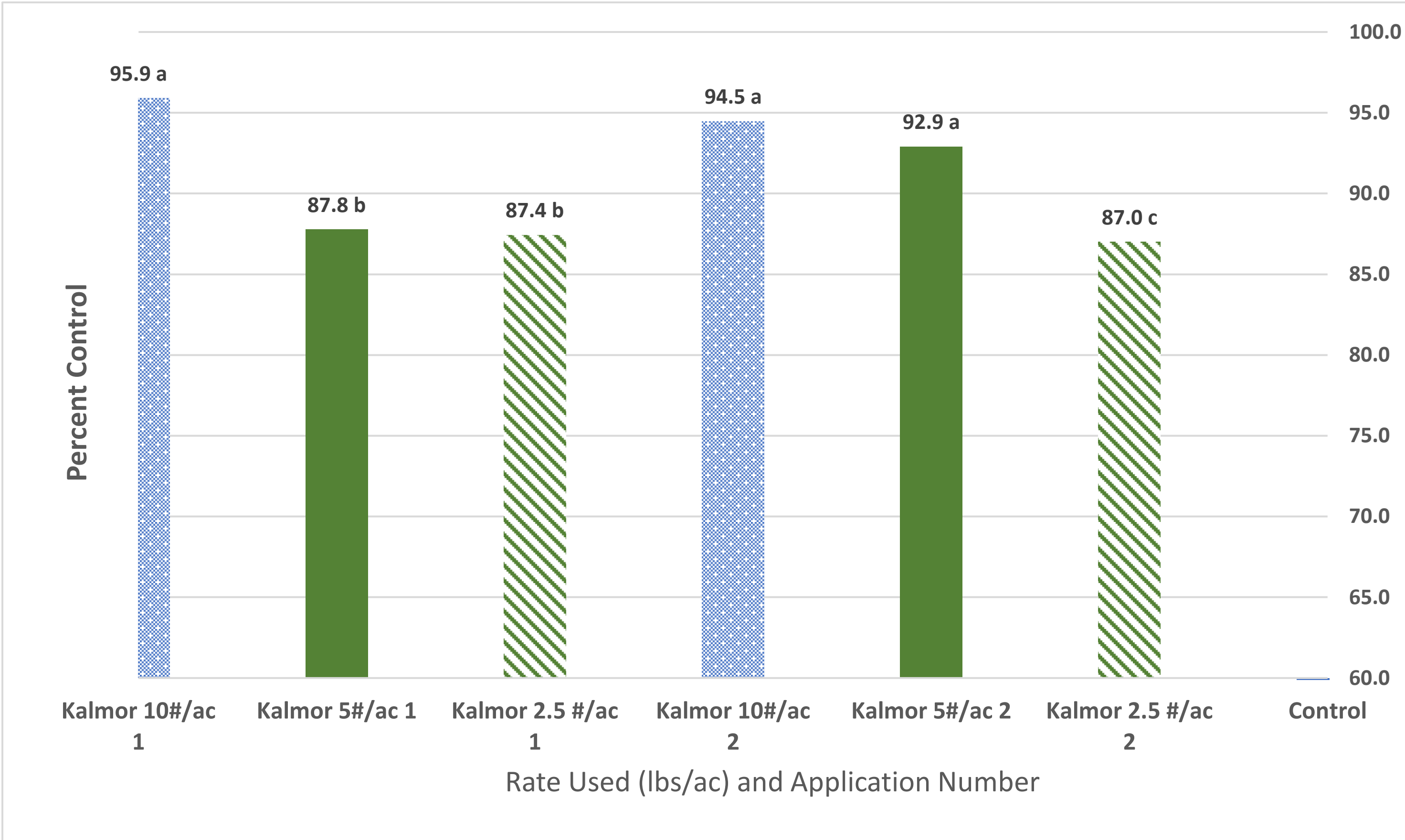


Fig. 4. The main effect of treatment for *Nostoc* sp. control. Each mean represents n=9, or three dates by three replications. The Kalmor® (EPA Reg. No. 91411-TX-001) (Copper hydroxice* 46.1%) (*Metallic copper equivalent 30%) (OHP, Inc., Bluffton, SC) trial was initiated on June 24, 2019 at Acorn Farms, Inc. (Wholesale Nursery), Galena, OH. Treatments with different letters signify efficacy was statistically different at p=0.05 using LS means following ANOVA in SAS.

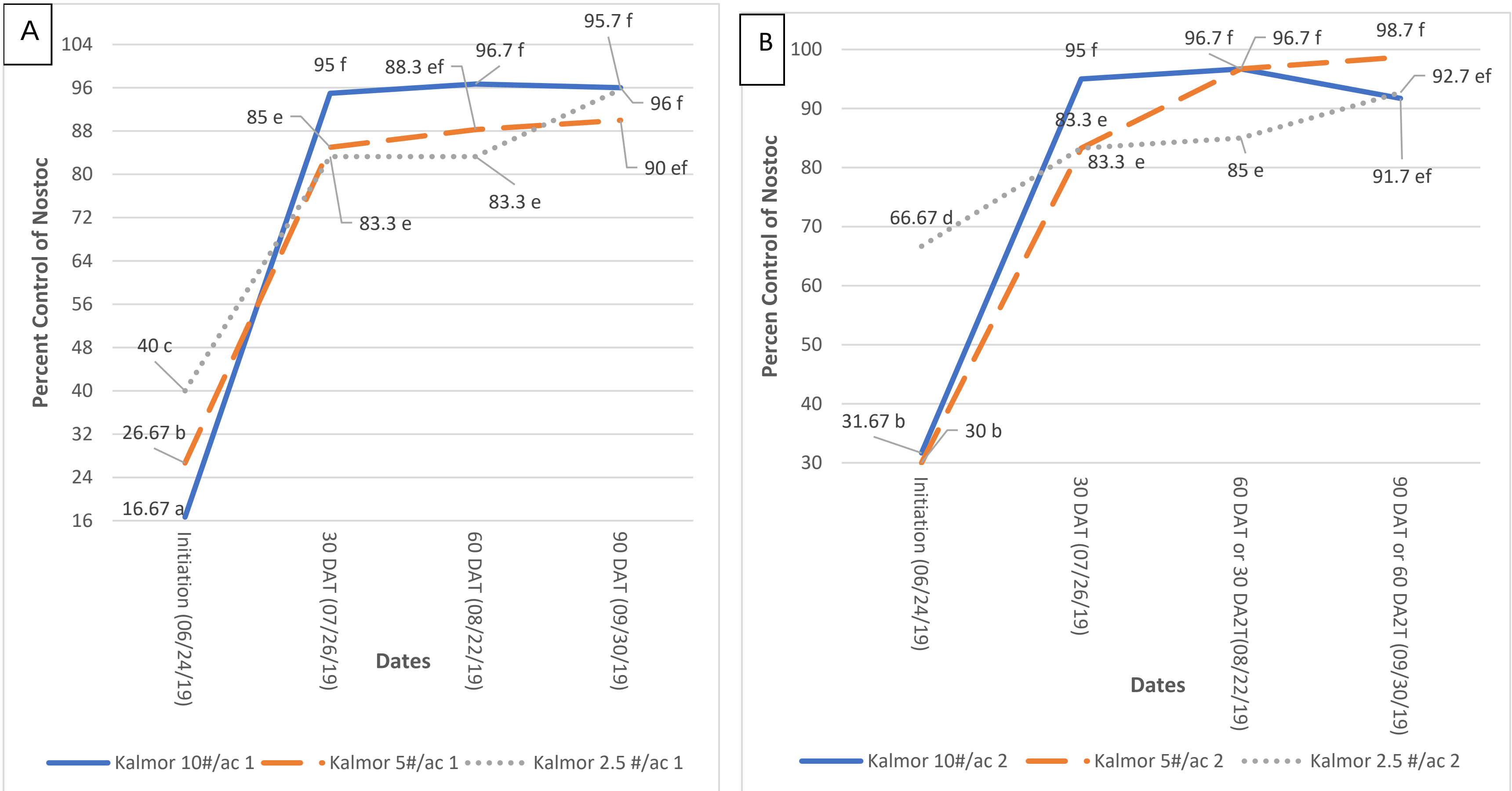


Fig. 5 A and B. The interaction effect of treatment by date for *Nostoc* sp. control. Each mean represents n=3, three replications. The Kalmor® (EPA Reg. No. 91411-TX-001) (Copper hydroxice* 46.1%) (*Metallic copper equivalent 30%) (OHP, Inc., Bluffton, SC) trial was initiated on June 24, 2019 at Acorn Farms, Inc. (Wholesale Nursery), Galena, OH. The interaction is separted into two garphs (A) recive one application abd (B) received two applications. The same rates were used in garph A and B. Treatments with different letters signify efficacy was statistically different at p=0.05 using LS means following ANOVA in SAS.

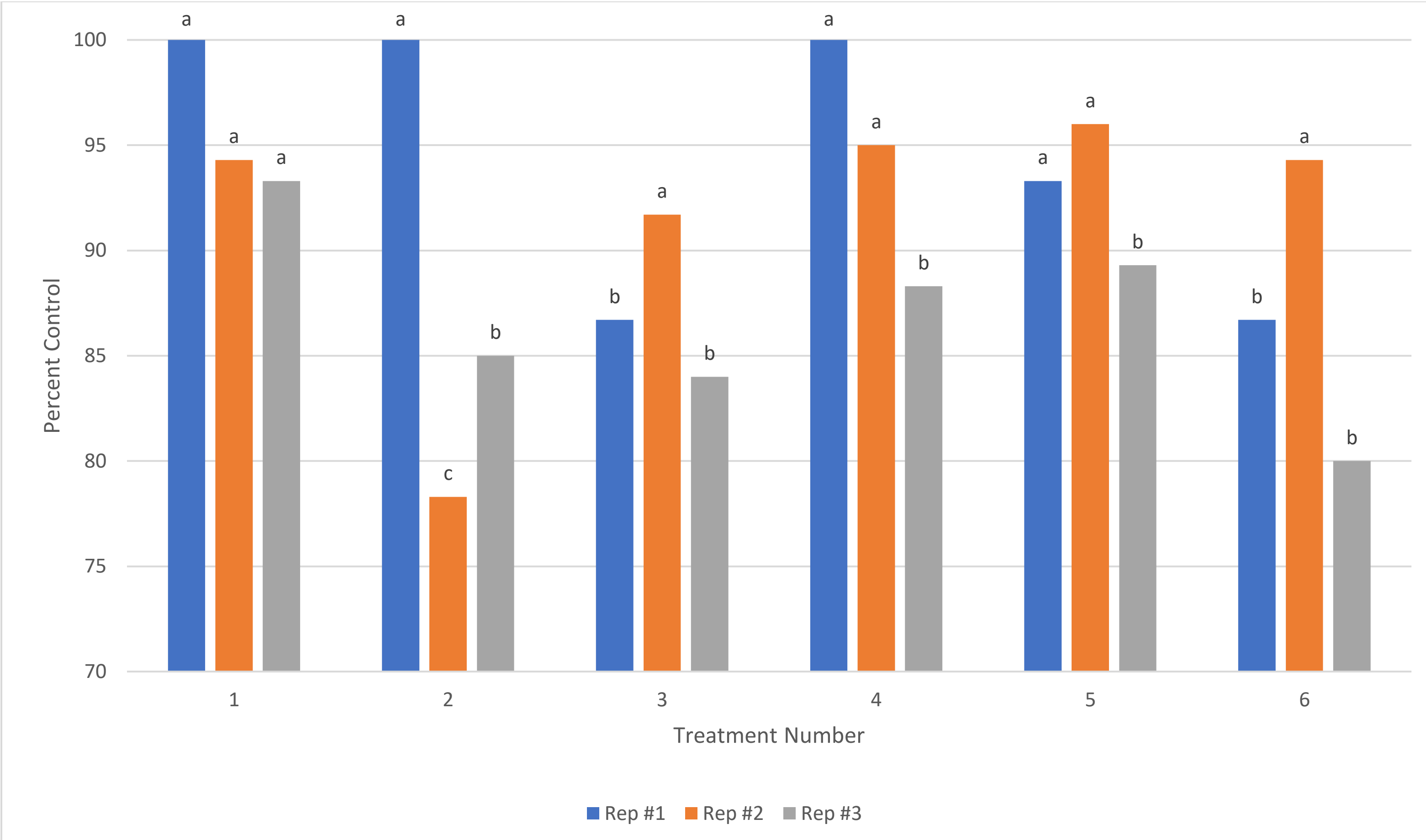


Fig. 6. The interaction effect of replication by treatment for *Nostoc* sp. control. Each mean represents n=3, three dates. The Kalmor® (EPA Reg. No. 91411-TX-001) (Copper hydroxice* 46.1%) (*Metallic copper equivalent 30%) (OHP, Inc., Bluffton, SC) trial was initiated on June 24, 2019 at Acorn Farms, Inc. (Wholesale Nursery), Galena, OH. Treatments with different letters signify efficacy was statistically different at p=0.05 using LS means following ANOVA in SAS.

Fig. 7. Replication three for *Nostoc* sp. control trial at Acorn Farms, Inc. (Wholesale Nursery), Galena, OH. The trial was initiated on June 24, 2019 and the photo is taken on 08/22/2019 or 60 DAT (days after the first treatment) or 30 DA2T (days after the second treatment). Shown is the path that the irrigation drainage water takes through the area including flowing through at least 25% to 50% of the right sides of the plots. The path of re-inoculation of *Nostoc,* sediments and algae are evident as it streams (Fig. 7). The area was saturated and difficult to work in without sinking. (Picture by: Dr. H. Mathers)

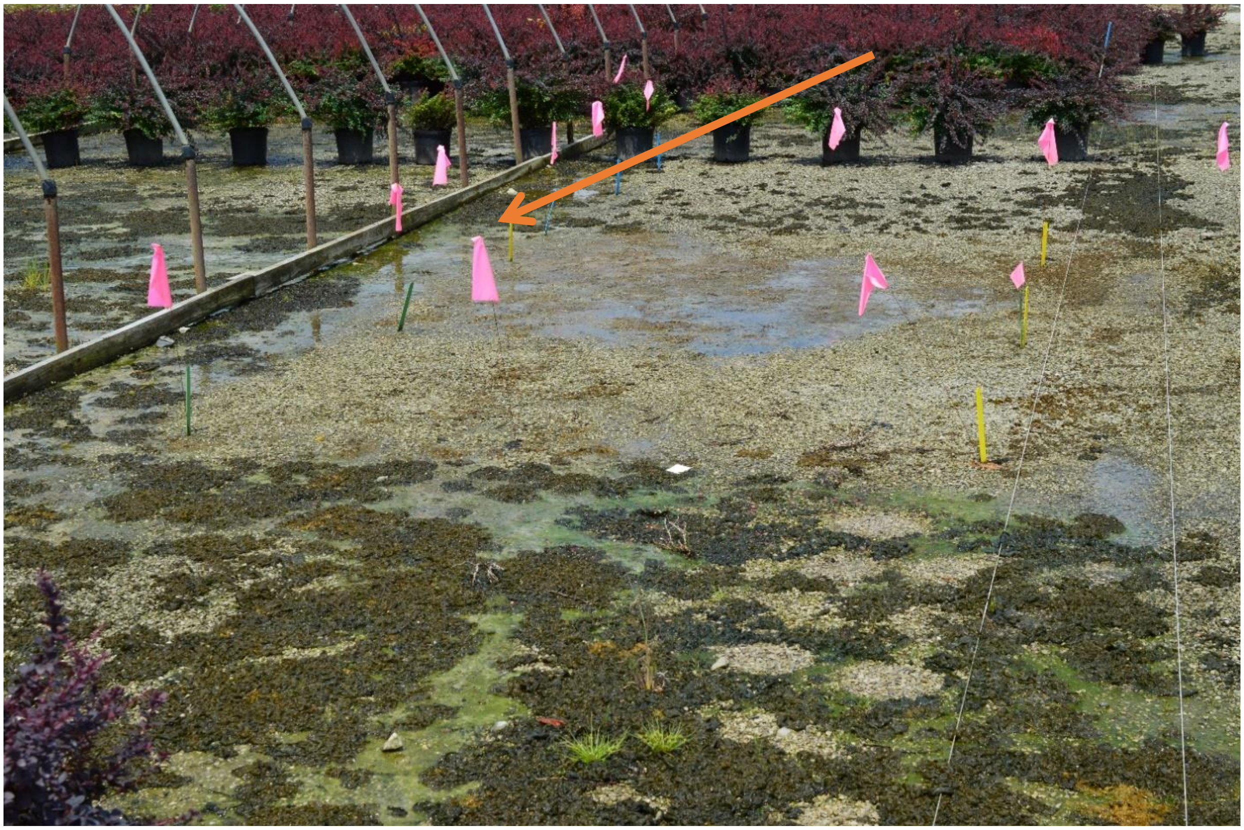


Fig. 8. (Above). The area shown comprised part of replication two at Acorn Farms, Galena, OH, taken on July 28, 2019 (4 WAT) as part of the *Nostoc* control trial. The area sprayed by Kalmor in the background, is easily delineated from the unsprayed area in the foreground. Treatment 1, 4, 2 and 5 are shown. An area of treatment 6 is shown right up against the hoop house foundation, towards the top of the treated area (labelled with orange arrow). Replication 2 had standing water however, irrigation drainage water was not flowing through the site as it was in replication 3. (Picture by: Dr. H. Mathers)

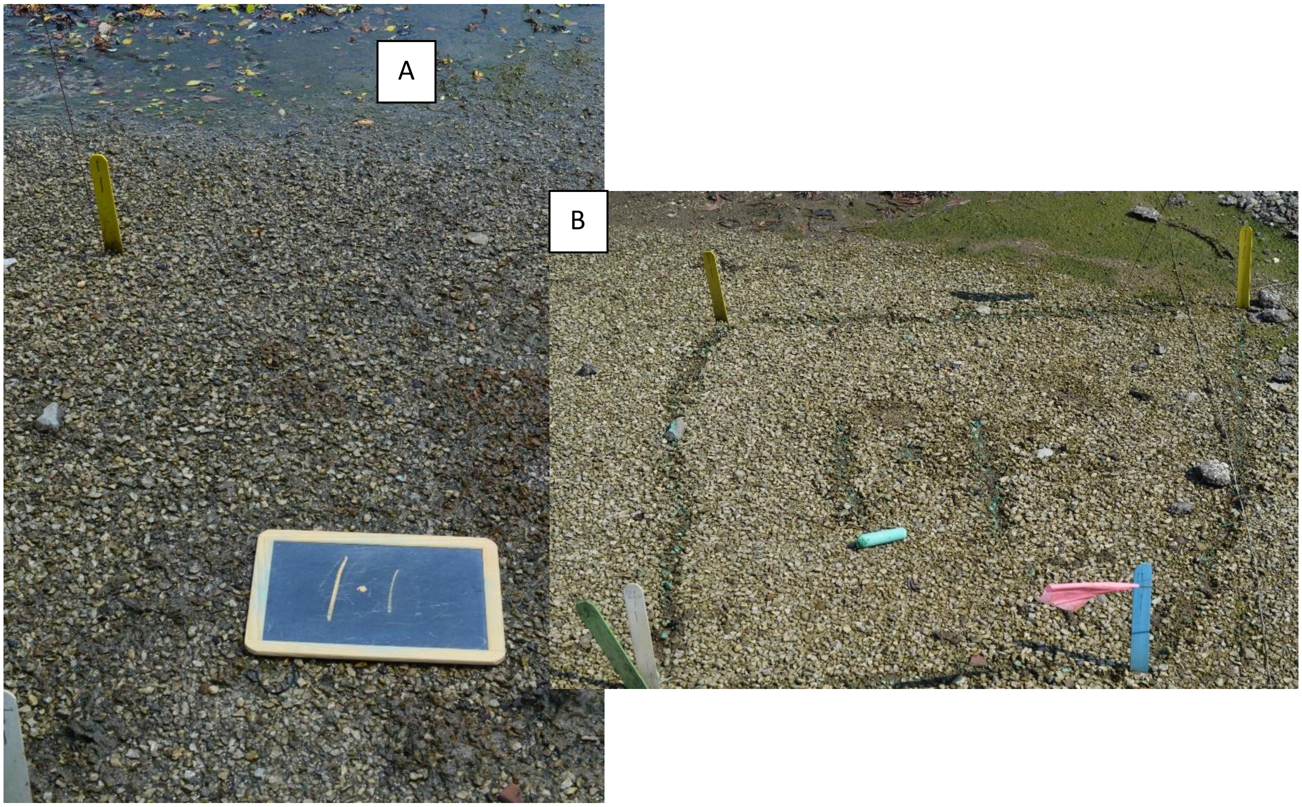


Fig. 9 A. B. C. and D. Treatment one (top) and four (below) in replication one at Acorn Farms, Galena, OH. **A** and **C** were taken at 30 DAT and **B** was taken at 90 DAT (09/30/19) and **D** at (60 DA2T). Nostoc control in both treatments is 100% at 09/30/2019 or the trial conclusion.

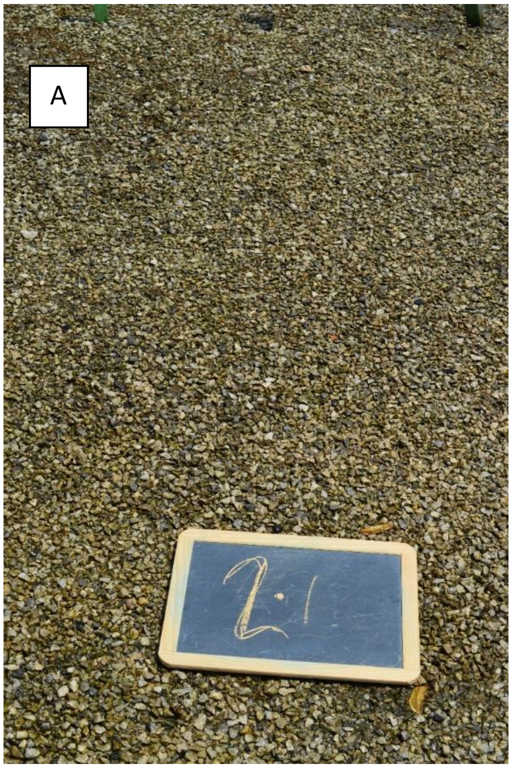
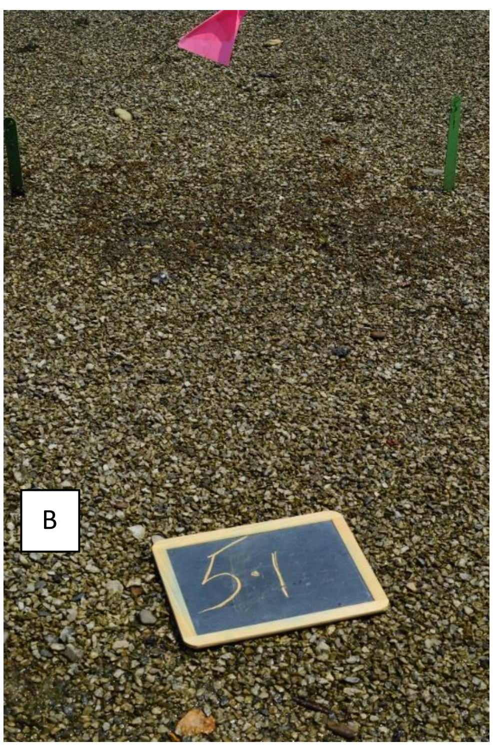


Fig. 10 A. B. and C. A. Treatment two (top) and **B.** five (below) in replication one at Acorn Farms, Galena, OH. **A** and **B** were taken at 30 DAT and **C** was taken at 90 DAT (09/30/19), respectively. **C.** *Nostoc* control in treatments 2 and 5 is 100% at 09/30/2019 or the trial conclusion. **C.** Treatment 2 and 5 are positioned side by side, with 5 to the right of 2. (Pictures by: Dr. H. Mathers)



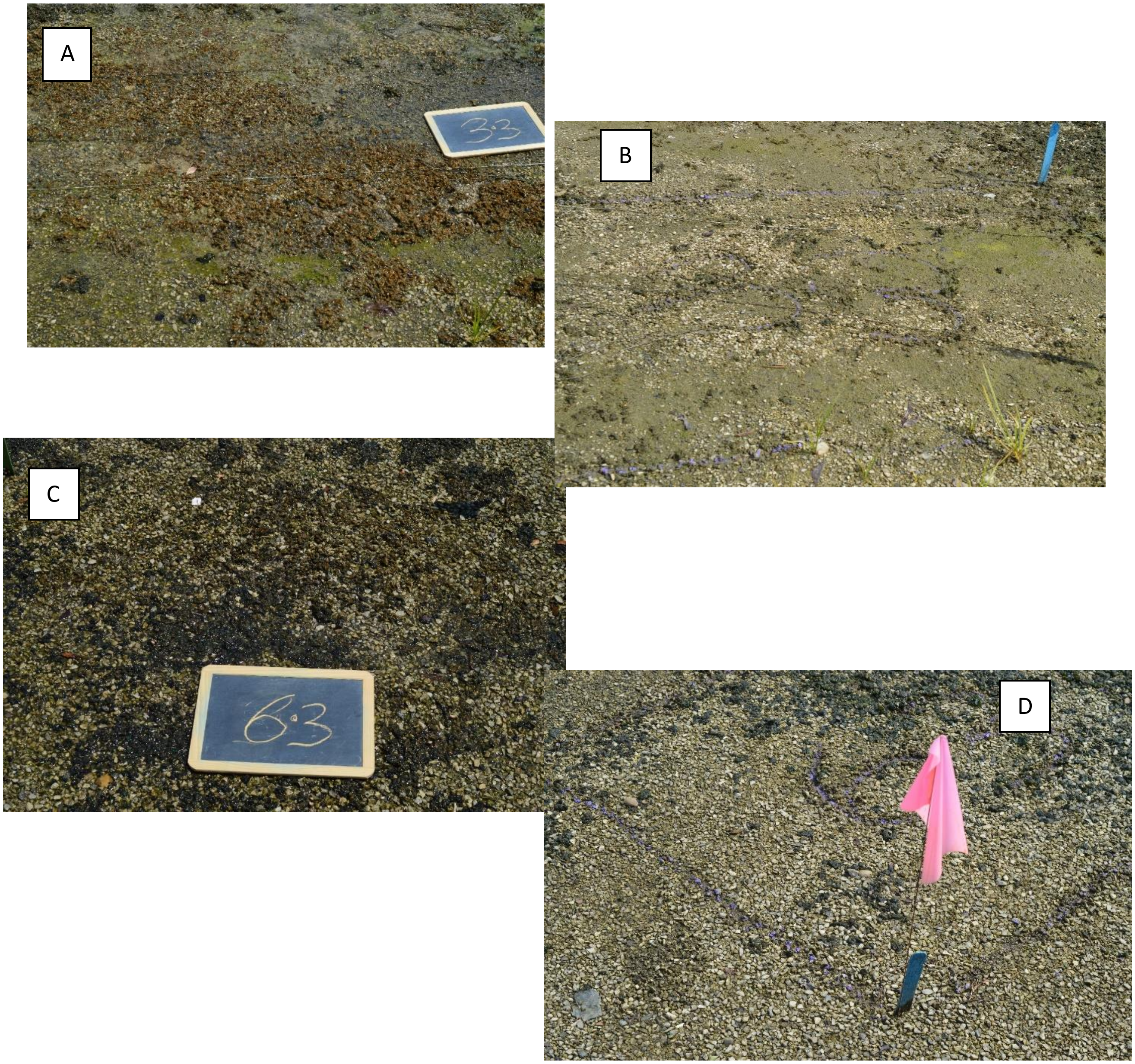
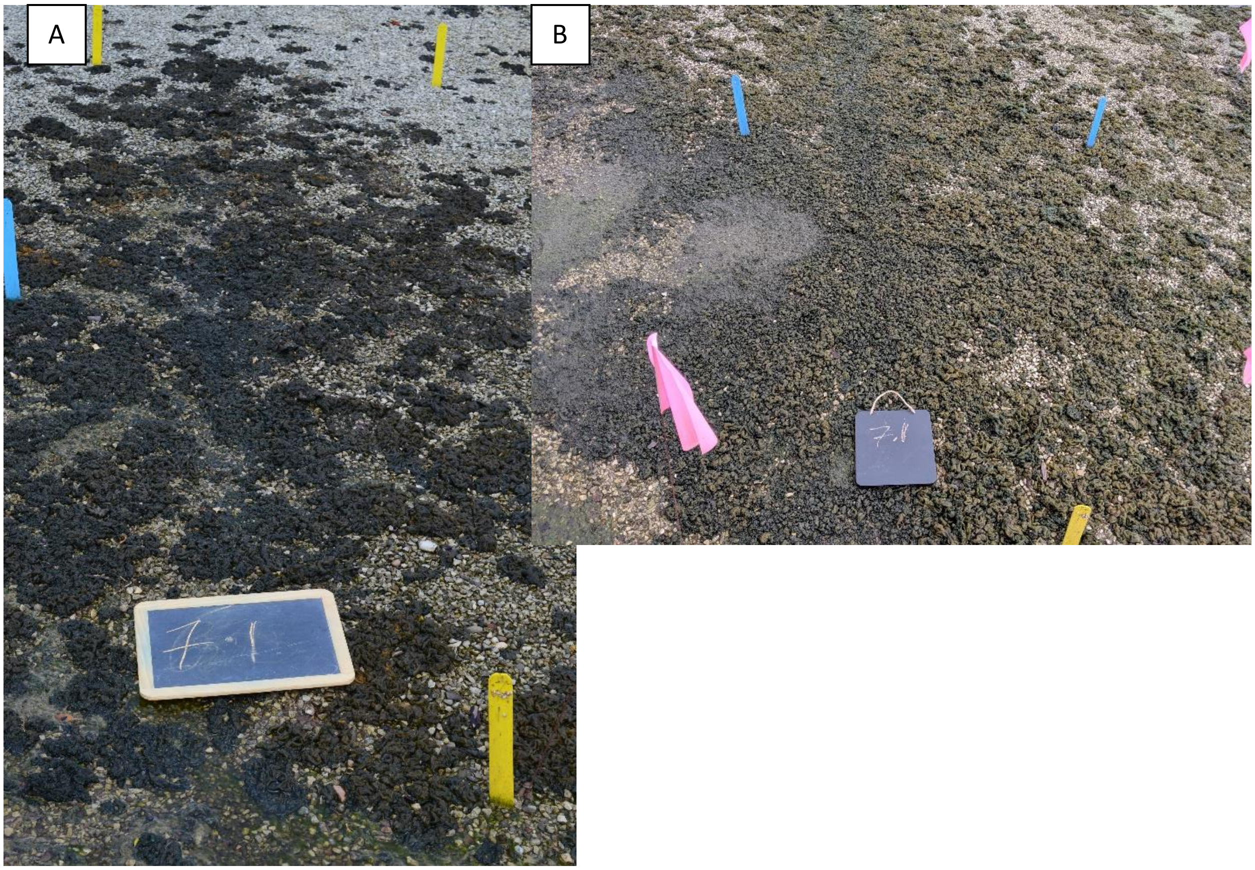


Fig. 11 A. B. C. and D. A. Treatment three (top), and **C**. treatment six (below) in replication three at Acorn Farms, Galena, OH. **A** and **C** were taken at 30 DAT and **B** was taken at 90 DAT (09/30/19) and **D** at (60 DA2T) (09/30/2019). **B.** *Nostoc* control in treatment 3 on 09/30/2019 is 92% at 09/30/2019. Significant debris and a few scattered patches of *Nostoc* were visible in treatment 3. **D.** *Nostoc* control in treatment 6 on 09/30/2019 is 80% at 09/30/2019. 20% of the area is still covered by *Nostoc*. Replicate three had the poorest control of *Nostoc* in general as irrigation drainage water flowed through part of the plots. Treatment 3 and 6, although not significantly different in control from treatments 1 and 4 (Fig. 9) and 2 and 5 (Fig. 10) when pooled over replication (Fig. 5), treatment 3 and 6 did not perform as well in replication 3, as in replication 1 and 2 (Fig. 6).



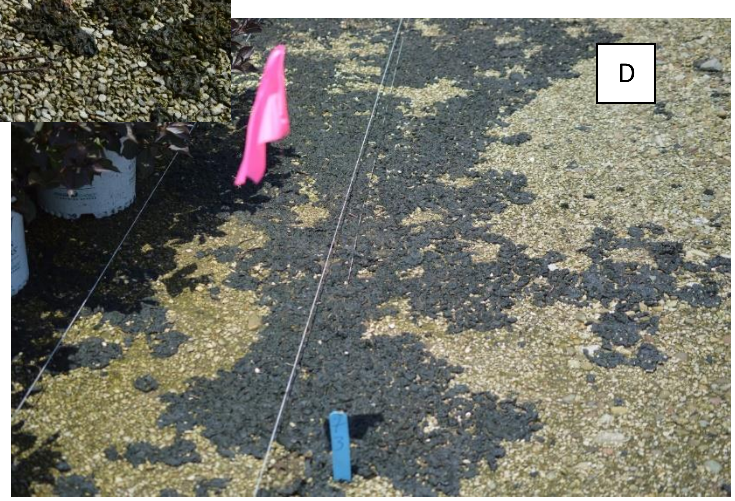


Fig. 12. A. B. and C. A. Treatment 7 (control) in replicate 1 at initiation and **B.** Treatment 7 in August 22, 2019 **i**n replicate 1. **C.** Treatment 7 in replicate 2 (4 WAT). **D.** Treatment 7 in replicate 3 at 4 WAT. There was no change in the controls, except a slight increase as in **B**. (Pictures by: Dr. H. Mathers)

Fig. 13. A. B and C. Treatment four, replicate three, at initiation of trial, June 24, 2019. **B.** Treatment 4, replicate three, at 4 WAT, July 28, 2019. **C.** Treatment 4, replicate three, at trial conclusion 60 DA2T (09/30/2014). Treatment 4 in replication 3, was one of the least efficacious treatments in the study at only 75% control on 09/30/2019. We speculated that the resurgence of *Nostoc* by the trail completion, in this replication, was due to the flow of drainage water through this replication, which its highest flow in this treatment plot. This replications decline caused the slight drop in control found with the 10 lb/ac rate polled over replications (Fig. 5 B). (Picture by: Dr. H. Mathers)

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