

# *Gunnera* at Cloudbridge Nature Reserve

Clarice Esch  
Western Kentucky University, Agriculture Department

## **Introduction**

Synthetic fertilizers are a critical component of agricultural production systems around the world. Nitrogen is a key ingredient, along with phosphorus and potassium, because they are vital for plant health and growth. Plants lacking nitrogen have limited growth and reproduction. The need to produce greater crop yields from less land leads many to apply nitrogen fertilizers in excess, polluting waterways with an overwhelming amount of nutrients, fueling toxic algal blooms, and creating oxygen dead zones.

In an effort to avoid the legal and environmental repercussions of polluting waterways, alternative fertilizers have become highly desirable, particularly in the greenhouse industry. Organic, sustainable and controllable forms of nitrogen are being sought out and examined in great detail, such as the legume rhizobium association. One unique option that has been limitedly studied is the symbiosis of *Gunnera* and *Nostoc*, a cyanobacteria. It is a true symbiosis in which *Nostoc* fixes nitrogen for *Gunnera* and *Gunnera* makes photosynthates available to support *Nostoc* (Silvester and Smith, 1969). Via this symbiosis *Nostoc* supports all of the nitrogen needs of *Gunnera*. This is the only symbiosis between an angiosperm and cyanobacteria (Osborne et al., 1992). With such a sophisticated endosymbiotic relationship it is remarkable that the relationship of *Gunnera* and *Nostoc* has gained so little attention in the realm of scientific research.

However in the world of aesthetics, the large tropical species of *Gunnera* have attracted horticulturalists for centuries because of their immense size, and contrast with most traditional landscapes (Image 1, lower right corner-*Gunnera manicata*). *Gunnera* is of an ancient angiosperm lineage, often classified into the Haloragaceae (watermilfoil) family, a family of aquatic plants. This placement has been debated. Although *Gunnera* does display traits indicating it is a

Image 1



transitional plant between aquatic and land plants, ie polystelic stem anatomy, it has been removed from the Haloragaceae family. *Gunnera* is now classified as belonging to the family Gunneraceae, a family with a single genus and seven subgenera (Bergman et al., 1992).

*Gunnera* is recognized by its single unbranched petiole emerging from a ligule-covered base at soil level.

The leaves range in size from a few centimeters to three meters across and are often described as rhubarb-like thus creating the common name, Giant Rhubarb. It has also been dubbed Poor Man's Umbrella for its large umbrella like leaves. Generally, *Gunnera* plants are rhizomatous or stoloniferous (Bergman et al., 1992). Flowers are produced by the hundreds if not thousands on large inflorescence spikes.

Among horticulturists, *Gunnera* is notoriously difficult to grow because of its need for constant water. Thus, in a landscape it is often placed next to a pond or stream. In its natural landscape, *Gunnera* functions as a disturbance colonizer. Found as the first colonizer on landslides and along stream banks, in Costa Rica *Gunnera* has also expanded to colonizing man-made disturbances, road cuts (Palkovic, 1978).

The *Gunnera-Nostoc* symbiosis is initiated via the entrance of *Nostoc* through a gland(s) at the base of each petiole. After moving through the channels of the gland, *Nostoc* penetrates some of *Gunnera*'s cells, forming pockets of heterocyst rich tissue and begins fixing nitrogen. *Nostoc* only occupies approximately 1% of *Gunnera*'s tissue, but is able to support all of the nitrogen needs of *Gunnera* (Osborne et al., 1992). This is significant because it demonstrates that *Nostoc* is capable of fixing biologically significant quantities of nitrogen and transferring them to an external source. This lends favor to the theory that this type of endosymbiotic relationship can

be effectively applied and induced in agricultural crops to create cropping systems that do not require synthetic nitrogen fertilizer.

Before implementing such an application, one must first understand the process that allows the symbiosis of *Gunnera* and *Nostoc*, and *Gunnera*'s function ecologically. The relationship with *Nostoc* allows *Gunnera* to be a disturbance colonizer, because *Gunnera* is able to colonize the nitrogen-poor soils characteristic of disturbed sites where other plants cannot survive.

Cloudbridge Nature Reserve, in Chuma, Costa Rica is a suitable site to examine *Gunnera*'s ecological niche. One population of *Gunnera* is known to be present on a landslide within the reserve. Additionally, Rio Uran and Rio Chirripo converge within the reserve. Thus, there is an exceptional amount of stream-side and river-side land where *Gunnera* is expected to be found.

## **Methods**

### The Study Site

**Image 2**



A *Gunnera* population is located in Cloudbridge North alongside and regrowing on a recent landslide (Image 2). A second population was noted just outside of the reserve, also growing alongside of a recent landslide. However, due to the terrain this population was deemed inaccessible. All trails within the reserve were walked and at lookout points binoculars were used to scan for additional populations, none were seen.

The accessible population is transversed by a trail and directly downslope from a remaining triangle of pasture land surrounded by Cloudbridge. A stream passes through the population. A majority of the population lies between the stream and the landslide and uphill from the trail. Plants are scattered along the length of the stream until the stream reaches a private garden downhill from

the landslide. On the side of the landslide furthest from the stream the *Gunnera* populates the area less densely. Smaller plants are present and intermixed with grasses and ferns growing on the slope. Few plants are regrowing on the bare soil of the recent landslide.

Due to the limitations of having a single *Gunnera* population methods were revised and much of the data collected became qualitative. Sampling of the population was limited by accessibility. A small portion of the population remained inaccessible for the course of this study. However, this segment of the population was small and its exclusion had a minimal effect on the results.

Work begins with identifying the species of *Gunnera* in Cloudbridge. Two are present within Costa Rica, *Gunnera insignis* and *Gunnera talamanca*. Diagnostic characteristics are used to determine which species is present. These characteristics are, bifurcation of veins, depth of leaf lobing, ligule coloration and texture, prickle height and curvature, and various floral and inflorescence characteristics. The information used to make this identification is found in the article "A Hybrid of *Gunnera* from Costa Rica" (Palkovic, 1978). It is also known that *Gunnera talamanca* and *Gunnera insignis* freely hybridize, so species identification became quite crucial. Multiple plants were sampled to ensure an accurate identification.

The next step was to determine if *Nostoc* is present within this *Gunnera* population. Although it is understood that all *Gunnera* plants contain *Nostoc* in nature, because no one has ever reported not finding *Nostoc* (Palkovic, 1974), one must be certain. Tissue samples in the field were taken directly behind the entry glands from plants of varying sizes throughout the population. These were examined under the microscope to identify any symbionts if present.

Once it was determined if *Nostoc* is present within the plants of this *Gunnera* population, it was necessary to determine where *Nostoc* is present within *Gunnera*. Although it seems a fairly basic issue it has only been addressed to a limited degree in the published literature. It is known that *Nostoc* is present directly behind the entry glands and throughout the rhizome. Thus, an

examination of all *Gunnera* tissue, from rhizome to leaf was conducted to determine where *Nostoc* inhabits *Gunnera*. Three entire plants were destructively sampled and then five additional petioles with some rhizome tissue were removed as well. These were separated into rhizome, petiole and leaf tissue. The rhizome tissue was systematically sliced from the aboveground node to the furthest underground point, the growing tip. Adventitious roots were also examined. The petiole was divided into four equal length segments and sampled for *Nostoc*. Samplings of vein and non-vein tissue were selected from the leaves. All of these samples were examined macro and microscopically. Then nondestructive sampling continued in the field to add to the results of the destructive sampling. Tissue samples were removed from the petiole in vertical slices to reduce structural damage to the *Gunnera* petioles.

During the recent rainy season, the landslide associated with the *Gunnera* population at Cloudbridge slipped, eliminating any *Gunnera* growing on it. However, there are now *Gunnera* plants regrowing on the bare ground that remains after the landslide. This allowed the division of the population into two sub-populations, established plants and regrowth. Measurements of leaf dimensions, petiole height and number of petioles per plant were recorded for all plants regrowing and for a set of plants within the established sub-population.

While conducting other tasks, observations were continuously made. Evidence of herbivory and disease were noted and other plants growing with *Gunnera* identified. Visual comparisons of plants growing in different parts of the populations were conducted as well as examinations for rhizomes. Also, canopy coverage and slope direction were noted. Soil samples were also collected to test for organic matter and mineral content.

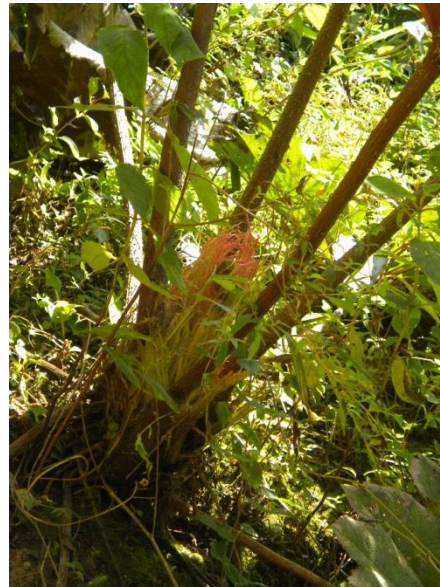
## **Results**

In the species identification of the *Gunnera* population all characteristics were based upon Palkovic's 1978 descriptions of *Gunnera insignis* and *Gunnera talamanca*. The primary veins of the leaves bifurcate at least twice, indicating *Gunnera insignis*. The lobing is shallow, another

characteristic of *Gunnera insignis* (Image 3). However, the ligules although pubescent from base to tip, an indication of *Gunnera insignis*, are not bright red as those of *G. insignis* should be.

Image 4

Image 3



Rather the ligules of the large plants are green in color with occasionally pink tips (Image 4), thus conforming to characteristics of *G. talamanca* or a possible hybrid. However, relatively young plants did show the characteristic red ligules. There are two *Gunnera* plants in the Cloudbridge gardens that were removed from this site and their ligules are red.

Regarding the reproductive portions of the plant, the branches of the inflorescence ranged from 2 to 30 cm depending upon the development and portion of the inflorescence the branch was removed from. This exceptionally long length indicates *G. insignis*. The bracts of the inflorescence, although not brick red as expected of *G. insignis* are pubescent and conform to the dimensions of *G. insignis*. All flowers sampled were bisexual with solitary sepals that were not lobed, further indicating that these plants are *G. insignis*.

Several plants of varying sizes throughout the population were sampled in the field and found to contain pockets of green tissue (Image 5), assumed to be *Nostoc*. Samples were brought back to the lab, dissected and examined under the microscope at 40x, the maximum

magnification available. The green tissue was able to be identified as *Nostoc* because of its distinctive green filaments and heterocysts.

**Image 5**



Samplings of entire *Gunnera* plants indicate that macroscopically, *Nostoc* is present in the rhizome but not within the petiole or leaf. *Nostoc* tissue was clearly visible throughout the rhizome from the aboveground node to the growing tip. No *Nostoc* was found in the adventitious roots.

Underground sampling of the rhizome was limited to smaller plants, *Nostoc* was uniformly present throughout the entire rhizome. Microscopically, *Nostoc* was not visible within the petioles, leaves or adventitious roots. Magnification was limited to 40x, thus it is very possible that *Nostoc* is present in the tissues but unidentified due to the limitations of the user and equipment. Further tissue samples confirmed the macroscopic findings that *Nostoc* is present in only the rhizome.

The comparison of established and regrowing populations provided limited information. Overall, the relationship between the petiole length and dimensions of the leaf demonstrated plants in the regrowing population had significantly shorter petioles relative to their leaf dimensions. However, this could be the result of many factors.

### Significant Observations

Much of this paper gives the impression that *G. insignis* and *G. talamanca* have rhizomes. However, prior to reaching the study site it was unknown whether or not rhizomes were present. General literature regarding *Gunnera* species worldwide reports that most species are rhizomatous or stoloniferous and that the large tropical species are rhizomatous (Bergman et al., 1992). Reports regarding *G. insignis* and *G. talamanca* specifically do not mention the presence of a rhizome (Palkovic, 1974; Palkovic, 1978). Although it is not surprising to discover that

rhizomes are present in these plants, it is significant because prior work has not reported their existence.

Some small plants that were destructively sampled to determine *Nostoc*'s location did not have rhizomes. These plants also demonstrated a slightly different leaf shape from the larger plants although the venation remains the same. The lobing of these leaves is less distinctive. (Images 6 (juvenile form) & 7 (mature form)). These anomalous features and lack of rhizome development suggest that these are juvenile forms of *Gunnera* plants, indicating that there is seed based reproduction occurring in this population.

**Image 6**



**Image 7**



When investigating the plants regrowing on the landslide all were determined to be growing via a rhizome. It is probable that during the landslide the rhizomes were severed and resulted in vegetative propagation. All of the regrowing plants were found lower than the trail.

**Image 8**



Petioles of the large *Gunnera* plants were noted to have small dark holes occasionally (Image 8). When sampling for the presence of *Nostoc*, cavities were noted within the petiole tissue. These cavities were found in conjunction with the holes visible to the exterior. Further sampling identified that the holes and cavities were



consistently found together. This may be the result of a burrowing insect.

Leaves were noted to be brittle and easily damaged. Many show signs of insect damage.

Image 9

Some were so severely damaged that only the veins and very little leaf tissue remain (Image 9). Most of the damage has occurred on plants on the south facing portion of the population, the part of the population furthest from the stream. Also, a single instance of apparent herbivory was observed in the removal of an emerging leaf. The markings on the remainder of the leaf indicate that it was bitten off, not cut by a machete.



The population occupies a slightly u-shaped slope. The majority of the population lies on the south west facing portion of the slope, however small pockets, those furthest from the stream, face south. Numerous grasses, ferns and *Piper umbellatum* were found growing alongside *Gunnera*. An analysis of the soil mineral and organic matter content has yet to be processed by the soils lab. Visual analysis indicates that it is a clay soil with little organic matter.

## Conclusions

*Gunnera* plants present at Cloudbridge Nature Reserve have been identified as *Gunnera insignis*, although the coloration of the ligules and floral bracts remained inconsistent with characteristics for *G. insignis*. Descriptions of *G. insignis* do indicate that a single population with nearly white colored ligules is present in Costa Rica (Palkovic, 1974). Since some of the small plants, which were well exposed to the sun, and the *Gunnera* plants transplanted to the Cloudbridge garden present red ligules, I suggest that the ligule coloration is dependent upon sun exposure. Plants with green ligules were in dense mats, thus the leaves shaded the ligules throughout the entire day. The smaller plants in the population were in full sun and unable to

shade their ligules and the *Gunnera* transplants, although large, are now solitary and in an open space which allowed only minimal shading.

Understanding where *Nostoc* inhabits *Gunnera* aids my continuing work greatly as I grow *Gunnera* plants at Western Kentucky University. I am now able to determine if the plants are infected with *Nostoc* through a single minimally destructive slice. Macroscopically, *Nostoc*'s limited penetration of *Gunnera*'s tissue (no deeper than 2 cm) indicates that once initiating intracellular inhabitation of *Gunnera*'s cells, *Nostoc* does not continue to colonize cells extensively. It is likely this applies on the microscopic level as well, thus making it unlikely that *Nostoc* would have been found at the microscopic level in the petiole or leaf tissue.

Distinguishing the juvenile leaf form assists with further field studies. Now an identification, at least among small plants, of sexually versus vegetatively propagated is a matter of appearance rather than disturbing the rhizome. Thus, one can make a loose determination of how much new growth is seed based, which indicates new genetics and can imply relative population health. As a wind pollinated plant, selfing is common among *Gunnera* plants (Palkovic, 1978). However, minimal genetic variation is still created via selfing. The miniscule size and immense quantity of *Gunnera*'s seeds indicates minimal investment in seed production. It is apparent that vegetative reproduction is *Gunnera*'s main method of populating an area, whether through a landslide severing the rhizome, or producing additional petioles at another node along the rhizome.

Both populations of *Gunnera*, the accessible and inaccessible population, were found on south facing slopes, indicating *Gunnera*'s immense need for sunlight and possible tolerance of conditions that would be too harsh for other plants. No shading is available to the *Gunnera* population from trees, only *Gunnera* leaves shading other leaves. *Gunnera* remains highly reliant upon a water source, which is expected with its semi-aquatic classification.

The combination of all this information indicates that *Gunnera* is not an excellent competitor, rather an opportunist. *Gunnera* produces massive quantities of seeds in hopes that a single seed will arrive in a favorable location. Then once established *Gunnera* is able to persist via large rhizomes and reproduce vegetatively through the assistance of disturbances on landslides or stream banks. *Gunnera* is certainly an opportunist based upon this information and serves as a colonizer of disturbed sites that other plants would find unfavorable. The *Nostoc* makes *Gunnera* a competitive colonizer by providing a nitrogen advantage. But, once other plants colonize disturbed sites behind *Gunnera* and begin adding organic matter to the soil as they decompose *Gunnera*'s advantage is lost.

### **Future Work at Cloudbridge**

A continuation of this work at Cloudbridge would begin with a closer examination of the riversides for *Gunnera* populations. Also, travelling up the valleys containing large tributaries could provide additional populations. To clarify the microscopic results regarding *Nostoc*'s occupancy of *Gunnera* a microscope capable of higher magnification would be used.

Future studies could compare the rate of regrowth on the landslide, assuming that another landslide does not occur. It would also be interesting to determine whether the population is expanding towards the forest, or the population is growing smaller and being pressed in by the forest. Over numerous years the progression of *Gunnera*'s growth or depression as a population could be easily examined and in this case used as an example of the length of time it takes a landslide to stabilize and the succession of colonizers to grow. Also, an analysis of *Gunnera*'s ability to stabilize the soil would be worthwhile, particularly because of its occurrence on road cuts.

## Work Cited

Bergman, B., C. Johansson, and E. Soderback. 1992. Tansley review no 42. the *Nostoc-Gunnera* symbiosis. *New Phytologist*. 122: 379-400.

Osborne, B. A., A. Cullen, P. Jones, and G. Campbell. 1992. Use of nitrogen by the *Nostoc-Gunnera tinctoria* (Molina) Mirbel symbiosis. *New Phytologist*. 120: 481-487.

Osborne, B. A., F. Doris, A. Cullen, R. McDonald, G. Campbell, and M. Steer. 1991. *Gunnera tinctoria*: an unusual nitrogen-fixing invader. *BioScience*. 41(4): 224-234.

Palkovic, L. 1978. A hybrid of *Gunnera* from Costa Rica. *Systematic Botany*. 3(2): 226-235.

Palkovic, L. 1974. The genealogy of the Genus *Gunnera* in Mexico and Central America. Harvard University, Cambridge, Massachusetts, PhD Diss. 42-72.

Silvester, W. B., and D. R. Smith. 1969. Nitrogen fixation by the *Gunnera-Nostoc* symbiosis. *Nature*. 224: 1231.