Sequestration and Release of Nutrients from Mist by Epiphytic Mosses and Orchids

Lauren Bennett

Department of Biology, the Colorado College

ABSTRACT

Epiphytes sequester required nutrients and moisture from mist; the remaining moisture is then released as throughfall for other organisms. I proposed that there were differences between types of cloud forest epiphytes in the amounts of nutrients that they sequester. Using mist collectors filled with three different types of epiphytes (mosses, orchids without pseudobulbs *Pleurothallis ruscifolia*, and orchids with pseudobulbs *Encyclia pseudopygmaea*) and a control (empty) mist collector, I compared concentration of mist that passed through the control collector and three epiphyte groups throughfall of volume, nitrate concentration, phosphate concentration and pH level. I found no significant difference in the volume, nitrate concentration or pH levels in the throughfall of epiphyte groups (Kruskal-Wallis, H = 3.16, P = 0.37; H = 1.46, P = 0.69; H = 7.49, P = 0.06 respectively). There was a significant difference among the phosphate concentration of the mist and *P. ruscifolia* (Kruskal-Wallis, H = 6.56, P = 0.04 and Tukey's test, Q = 2.43, P = <0.05), indicating these orchids leach phosphate. The concentration of nitrate was positively related to the volume of water collected (simple regression, R^2 = 0.57, P = 0.04). There are many possible unstudied atmospheric conditions could account for this relationship. The concentration of nitrate was significantly greater immediately following a drought in comparison to days with normal precipitation (one-sample t- test, t = -14.67, P < 0.01, hypothesized mean = 1.36 ppm), following the known trend that atmospheric ion concentrations are greater at the end of the dry season, and are found in high concentrations following normal rainfall. Using a second study site I collected mist to determine if there were differences in mist precipitation with altitude. There were two days that did not have matching mist precipitation between the two study sites, indicating a possible altitudinal difference.

RESUMEN

Las epífitas atrapan los nutrientes llevados por el rocío; la humedad restante entonces se libera y cae, haciéndose disponible para otros organismos. Se predijo que habría diferencias en la cantidad de nutrientes que los diferentes tipos de epífitas atrapan en el bosque nuboso. Se emplearon recolectores de neblina con tres tipos diferentes de epífitas (musgos, la orquídea sin seudobulbos *Pleurothallis ruscifolia* y la orquídea con seudobulbos *Encyclia pseudopygmaea*) y un colector de control vacío. Se compararon las concentraciones de neblina que pasaron a través del recolector de control y de los recolectores de tratamiento con respecto al volumen, a las concentraciones de nitrato, de fosfato y el valor de pH. No se encontraron diferencias significativas ni en la concentración del nitrato ni en el nivel de pH en los grupos de epífitas (Kruskal-Wallis, H = 3.16, P = 0.37; H = 1.46, P = 0.69; H = 7.49, P = 0.06, respectivamente). Se encontraron diferencias significativas entre las concentraciones de fosfato de la niebla en *P. ruscifolia* (Kruskal-Wallis, H = 6.56, P = 0.04 y la prueba de Tukey, Q = 2.43, P = <0.05), indicando que estas orquídeas gotean fosfato. La concentración de nitrato estuvo relacionada positivamente al volumen de agua (regresión sencilla, R^2 = 0.57, P = 0.04). Existen muchas condiciones atmosféricas que podría explicar esta relación y cuyas influencias no han sido consideradas. La concentración de nitrato fue significativamente mayor inmediatamente después de una sequía que en días con precipitación normal (prueba de t, T = -14.67, P <0.01, promedio hipotético = 1.36 ppm), siguiendo la tendencia conocida de que las concentraciones atmosféricas iónicas son mayores a finales de la temporada seca y se encuentran a altas concentraciones después de una lluvia normal. También se recolectó neblina en un segundo sitio para determinar si había diferencias en la precipitación de la niebla con la altitud. Se presentaron dos días en los que la precipitación no fue la misma entre los dos sitios de estudio, indicando una posible diferencia altitudinal.

INTRODUCTION

Epiphytes are plants that grow in on top of other plants. They depend on trees or other plants for their structural, but not nutritional support, and never have a direct connection to the ground (Ingram 2000). Many live in the upper reaches of the canopy. Instead of taking nutrients from the soil, these epiphytes use their roots and leaves to remove moisture from the air and rain, and sequester nutrients from the mist or dust in the canopy (Raven et al. 1999). This group of plants includes orchids, ferns, bromeliads, and bryophytes among others (Ingram 2000). Wet Neotropical forests such as the Monteverde Cloud Forest have a greater abundance of epiphytic species than any other forest type (Ingram 2000). This type of plant growth form covers much of the vegetation in the Monteverde Cloud Forest where mist and close cloud cover are frequent, and epiphytes are able to extract moisture and take up nutrients from the air, mist, rain and dust (Ingram 2000).

Due to variation in availability of moisture and nutrients, epiphytes must have special adaptations to ensure consistent adequate nutrition. Some epiphytes have adapted vascular structures for water storage (Ingram 2000). Non-vascular epiphytes have adapted to have increased surface area as a means of maximizing absorption (Nadkarni 1984).

Mist clearly plays an important role in the lives of epiphytes. Epiphytes depend heavily on the mist for the moisture and nutrients that they require to live. In tropical montane areas, an area frequently immersed in cloud cover, mist contributes a significant amount to the total ion deposition in cloud forests (Clark et al. 1998). Mist provides critical components necessary for epiphytes to survive; ion concentrations in mist are typically three to ten times greater than normal precipitation concentrations (Johnson and Lindberg 1992). Minerals held within epiphytes account for up to 45% of the total minerals in the foliage of the forest; epiphytes act as a buffer to nutrient pulses and retain moisture and nutrients for later use during times of drought (Nadkarni 1984). Past studies show that canopies retain inorganic nitrogen, while leeching phosphate and other nutrients, from mist and rain (Clark et al. 1998). Epiphytes are important elements in the forest that retain large quantities of nutrients in a nutrient poor environment, how these plants contribute to the cycle is an important connection.

Epiphytic organic matter is important in the nutrient cycles because it provides a large pool of carbon and other nutrients (Nadkarni et al. 2000). Nutrients from living and dead epiphytic matter is released into the terrestrially rooted material in three possible ways: epiphytic mats on host tree branches and trunks can be intertwined with host canopy tree roots, or the epiphyte mats become saturated and the remaining nutrients and moisture are considered mist throughfall and available for other plants to absorb, or finally epiphytic material can fall from the trees to the forest floor and decompose returning nutrients to the system (Nadkarni and Matelson 1992). Due to the lack of a waxy cuticle and large water storage capacity epiphytic bryophytes retain high levels of NO_3^- when located in the upper canopy (Clark et al. 2005). For a large epiphytic rich canopy community in a windy environment the water storage capacity is high and variable due to changing conditions (Clark et al. 1998)

The most abundant epiphytes are bryophytes, non-vascular plants, of which 50% are mosses that help provide substrate for other epiphytes to attach to (Gradstein 2000); together bryophytes make up between 5-20% of epiphytes (Ingram 2000). Another abundant group of epiphytes are members of the Orchidaceae or orchid family, these are vascular plants that are often found as epiphytic species (Atwood 2000) and when combined with the other angiosperm families together they comprise up to 80% of total epiphytes (Ingram 2000). Many of the epiphytic orchids have a layer of dead, spongy cells, known as the velamen, located on the outer surface of the roots, which service to hold and absorb water and nutrients (Dressler 1993). Some species of orchids have adapted a specialized thickened stem known as a pseudobulb which serves to store water and reduce drying (Dressler 1993).

Little information is currently known about the removal of nutrients by different species of epiphytes. I focus on the sequestering of nutrients by the epiphyte mats and collect the remaining moisture that would normally be released as throughfall to look at the nutrient removal. I use three different treatments of epiphytes: mosses, non-pseudobulb orchids *Pleurothallis ruscifolia* (Orchidaceae), and a pseudobulb orchid *Encyclia pseudopygmaea* (Orchidaceae). These different groups are compared against a control of pure mist. I look at the variation between nutrient removal from the mist of these three different groups and how much moisture is withdrawn by the epiphytes. I examine how nitrate (NO_3^-), phosphate (PO_4^-), and pH levels vary between the different treatments. I expect to find a difference in the volume collected from each treatment, with the mosses retaining the most water due to ratio of surface area, followed by *E. pseudopygmaea*, which would retain more water than *P. ruscifolia* due to the presence of pseudobulbs. I also expect to find that the *E. pseudopygmaea* will retain more nutrients than the other two treatments due to having pseudobulbs.

Since precipitation is needed for the survival of epiphytes, they are very susceptible to changes in precipitation (Gradstein 2000). A major question facing the world currently is how cloud cover is changing with global warming, and if there is a corresponding change in precipitation (Pounds et al. 2006). I used an additional lower altitude site to determine if there was a difference in number of days where mist was precipitated versus days that did not precipitate mist for the two locations, implying altitudinal variation in mist precipitation. I do not expect to find any difference in days that mist is precipitated between these two sites.

MATERIALS AND METHODS

The study was performed between April 18, 2006 and May 8, 2006 in Monteverde, Costa Rica on the Cerro Amigos, above the Estación Biológica de Monteverde (~1750 m). The site was completely exposed to the oncoming mist and oriented to the north to maximize exposure. An additional mist collector, used solely to compare the frequency of mist precipitation at upper and lower sites was set on the Estación Biológica de Monteverde property at approximately 1520 m.

The mist collectors were constructed using two Petri dishes as the top and bottom of the collector (Figure 1). Each dish had twenty-nine small holes drilled around the outside edge spaced as evenly as possible. The center of the collector was supported using a small section of PVC pipe that was 450 ml long. This was connected to the Petri dishes using a hot glue gun. The collectors were strung with thin gauge nylon fishing line connecting the holes on the top Petri dish to the bottom holes. A small loop was tied onto the top of the collector using the same fishing line and used to hang the mist collector. The bottom Petri dish of the collector was then attached to a collection funnel using hot glue. The base of the funnel was connected to a section of plastic tubing which runs into the 2 L collection bottle. These connections were secured using duct tape that was changed when samples were collected.

The setup consisted of a control collector, constructed in the same manner but not filled with any epiphyte group, which controlled for any additional removal of nutrients and volume by the collector itself, and served as a reference for what nutrients were actually present in the mist. The other collectors were filled with 0.62 g of mosses collected from the forest around the ridge test site, and filled with 0.62 g of *P. ruscifolia* and *E. pseudopygmaea*, which were collected from surrounding areas. The soil mat from the mosses, and orchids was removed from the roots of all of the epiphytes as much as possible, to eliminate any additional nutrient uptake or sequestration by the soil. The four collectors were set up on a frame directly facing the oncoming mist (Figure 1).

I checked the mist collectors between 11-11:30 am, either at 24-hour or 48-hour intervals. The volume in each bottle was measured using a 50 ml graduated cylinder and a sample of water from each collector and was brought back to the laboratory to analyze with a water testing kit (LaMotte water quality kit). Before performing chemical tests, I filtered each of the water samples to remove any solid matter picked up from the epiphyte treatments. I measured the nitrate concentration, phosphate concentration and pH level of each of the water samples. When there was not a sufficient volume of water collected to perform all of the tests I analyzed nitrate concentration first, phosphate concentration second and pH third. For each sample I took three readings and calculated the average of these. I recorded the volume of mist on 14 days, the sample size for the remaining treatments and tests varied from two to nine. I analyzed the data with a Kruskal-Wallis test (Stat View), and a Tukey's test as needed (Zar 1984).

I recorded the frequency of days on which measurable precipitation occurred at the Ridge site as well as the Estación site. I used this information to determine if there was altitudinal variation between sites where mist precipitated.

RESULTS

Mist was collected at the Ridge site on 12 of the 14 days I observed. There were often not adequate amounts of mist to measure all of the levels of nitrate, phosphate and pH. There was no significant difference found between the throughfall volume of the control collector and that of the epiphyte groups (Kruskal-Wallis, H = 3.16, P = 0.37); the volume of mist collected in the control over the 14 days ranged from 0.0 ml to 141.5 ml. There was also no statistical difference found between concentration of NO_3^- for the control collector and the epiphyte groups (Kruskal-Wallis, H = 1.46, P = 0.69); the average concentration of nitrate collected for pure mist was 0.37 ± 0.07 ppm (Table 1). In addition, I found no significant difference between pH levels for the control collector and the epiphyte groups (Kruskal-Wallis, H = 7.49, P = 0.06); the average pH of pure mist was 5.89 ± 0.09. However there was a statistical significance found between the concentration of PO_4^- for the control collector and the epiphyte groups (Kruskal-Wallis, H = 6.56, P = 0.04; Figure 2); the moss treatment only had a sample size of two, this data was excluded for the phosphate analysis and the remaining orchid treatments were tested. The concentration of PO_4^- in pure mist was 0.21 ± 0.04 ppm. I found that the *P. ruscifolia* treatment was significantly greater in PO_4^- than the control (Tukey's test, Q = 2.43, P = <0.05) the *P. ruscifolia* treatment had a mean PO_4^- concentration of 1.11 ± 0.35 ppm.

I also found that there was a significant positive relationship between volume of water collected and concentration of NO_3^- collected as throughfall in the control (simple linear regression, $R^2 = 0.57$, $P = 0.04$; Figure 3).

In addition to these findings I also noted that there was a significantly higher concentration of NO_3^- collected on the day immediately following drought than the other days with regular precipitation (one-sample t- test, $t = -14.67$, $P < 0.01$, hypothesized mean = 1.36 ppm).

While mist was collected 12 of the 14 days at the Ridge site, mist was collected for 10 of the 14 days observed at the Station site (Figure 4).

DISCUSSION

The results show that there is no significant difference in volume of mist collected between the different epiphyte groups (mosses, psuedobulb orchids, and non-pseudobulb orchids); this implies that all of the epiphytes removed the same volume of mist, and that this was not a great deal more than was actually precipitated. This seems counterintuitive; epiphytes would have to take up some amount of moisture to ensure survival. Possibly there was a difference but it was very small and therefore I was unable to measure it with the techniques I applied. The adaptations that I originally believed would aid in removal of water (increased surface area for the mosses and the pseudobulb for the *E. pseudopygmaea),* did not have a significant effect in aiding the epiphyte with sequestration of moisture. A further test that could be used to determine if this holds true is using larger masses of epiphytes to attempt and detect a difference.

The difference in NO_3^- collected was not significant between the different epiphyte groups and the control collector; this was not in agreement with the finding that forest canopies retain inorganic nitrogen (Johnson and Lindberg 1992, Clark et al. 2005), possibly a result of very limited sample size. Also there are other elements in the forest canopy, such as emergent trees, that may account for the retention of inorganic nitrogen previously found in other studies. The amount of nitrate in the mist found by prior studies was 0.47 ± 0.06 mg (Clark et al. 1998) and 1.19 mg (Clark et al. 2005), the values for nitrate mist concentrations are variable and may change due to the seasonality and yearly changes. These past studies were not measuring the differences between these specific species of epiphytes and instead looking at the whole canopy; collectively the epiphytes would retain more nitrate than measured on an individual basis. This is an important consideration for future studies.

The pH values were not significantly different between treatments. The mean pH of cloud mist found in previous studies was 4.32 (Clark et al. 1998), which was more acidic than the pH of the mist collected in this study, 5.89. Seasonal variation and regional differences can cause major variation in pH levels (Clark et al. 1998). This test would require a greater sample size over a longer period of time to determine if this developing trend holds true and determine the mean pH level for mist in this region. This test was very close to significant with such a small sample size; increasing the sample size would help determine if there is a difference, and to see if the mist in this location is the same approximate pH as the mist collected in other studies.

The PO_4^- concentration was found to be significantly different between treatments; the normal amount of phosphate in mist was 0.003 ± .001 mg (Clark et al. 1998). Specifically, the *P. ruscifolia* concentrations were greater than the control treatment indicating that they were leaching phosphates. This follows the pervious finding that phosphate leeches from the canopy (Clark et al. 1998). Possibly, with the lack of a soil mat or moss base for the roots of the *P. ruscifolia*, the orchids were unable to retain the nutrients and therefore leached them in the form of throughfall. It is important to examine if there is a mutualism between the orchids and the mosses for retention of nutrients. There are many components to nutrient cycling within the forest and understanding how each epiphyte adds or subtracts from the overall nutrient pool is necessary to comprehending the system function.

I began collection of mist immediately following an eight day drought; the concentration of NO_3^- in the first sample was significantly greater than the concentrations found on the following days. This study was conducted at the end of the dry season when regional practices of burning are greatest and concentrations of inorganic nitrogen are two to three folds greater than ion concentrations for the rest of the year (Clark et al. 1998, Clark et al. 2005). Therefore, the first rains would have very high ion concentrations that could bind to the mist and precipitate in the collector accounting for the high nitrate concentration found at the end of the prolonged dry period.

An additional finding was that the volume of precipitation increased the concentration of nitrate also increased. This seems counterintuitive due to the flushing out of ions with high precipitation and causing lower concentrations. However one possible untested reason for this finding was that during the days with high levels of precipitation the temperature was lower due to the increased cloud cover, possibly causing the nitrate to go into solution better than on warmer days with less precipitation and cloud cover. These hypotheses remain untested and deserve further study to determine the reasons for this trend.

With how dependent epiphytes are on mist, and with changing climate conditions it is important to understand if there are changes ongoing in this area. The two sites, Station and Ridge, had corresponding mist precipitation for 12 of the 14 collection days with the exception of two during which there was mist at the ridge site but not at the station site. This could be due to the altitudinal difference and concur with prior findings of increasing cloud cover in the Monteverde area due to global warming events (Pounds et al. 2006). A corresponding question with increased cloud cover is, is there a precipitation increase, or is this moving up the mountain with the clouds? This needs further study and examination for a longer period of time to see if these trends develop.

This study found that there are many different areas open for further examination. It is important to understand that these findings are currently only for the organism level and cannot yet be expanded to the ecosystem level. These findings show that there are different epiphytes that are adding to the nutrient cycle in different aspects, and that in order to determine how the ecosystem functions; many more plants need to be tested and examined for relationships between different species of plants. My study can be used to build up an analysis for the entire ecosystem once other epiphytes are examined we will be able to understand how all epiphytes contribute to nutrient cycling. The sample size was a limiting factor, both in volume of water and in number of days collected; it would be interesting to see if the same trends continue over a longer period of time and if the same trends are present in the wet season where precipitation is abundant. It would be interesting to focus on phosphate testing since that relationship was found to be significant and determine if there are differences in leeching rates with or without soil mats on the *P.*

ruscifolia, as well as determine if the trend with higher levels of nitrate precipitation immediately follows long dry periods.

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TABLE 1. Ridge site mist mean volume (A), nitrate concentration (B), phosphate concentration (C) and pH levels (D) collected per treatment.

A)	Volume (mL)			
	Control	Moss	*P. ruscifolia*	*E. pseudopygmaea*
Mean	38.21	16.65	21.88	28.65
SE	12.71	9.03	13.4	11.87
Sample size	14	13	13	13

B)	NO_3^- (ppm)			
	Control	Moss	*P. ruscifolia*	*E. pseudopygmaea*
Mean	0.37	0.12	0.38	0.26
SE	0.07	0.41	0.12	0.02
Sample size	9	6	6	6

C)	PO_4^- (ppm)			
	Control	Moss	*P. ruscifolia*	*E. pseudopygmaea*
Mean	0.21	1.43	1.11	0.21
SE	0.04	0.88	0.35	0.05
Sample size	7	2	4	5

D)	pH			
	Control	Moss	*P. ruscifolia*	*E. pseudopygmaea*
Mean	5.89	7.73	6.87	5.90
SE	0.09	6.87	0.69	0.10
Sample size	6	3	3	5

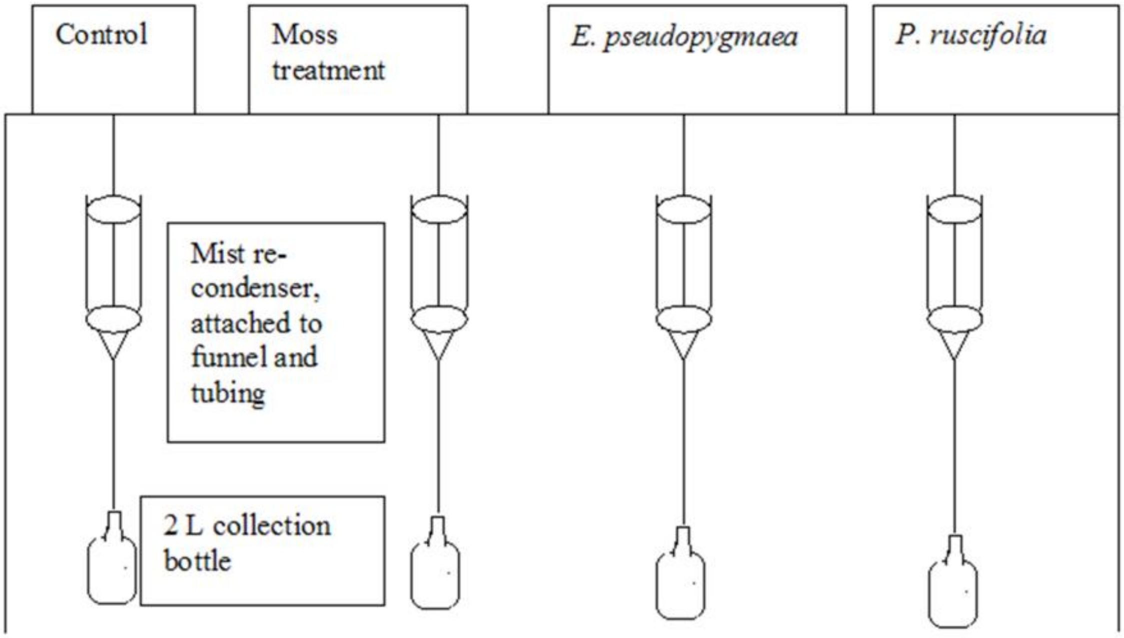

FIGURE 1. Cerro Amigos Ridge site experimental treatment setup. Mist collectors on frame, with collection bottles attached at bottom.

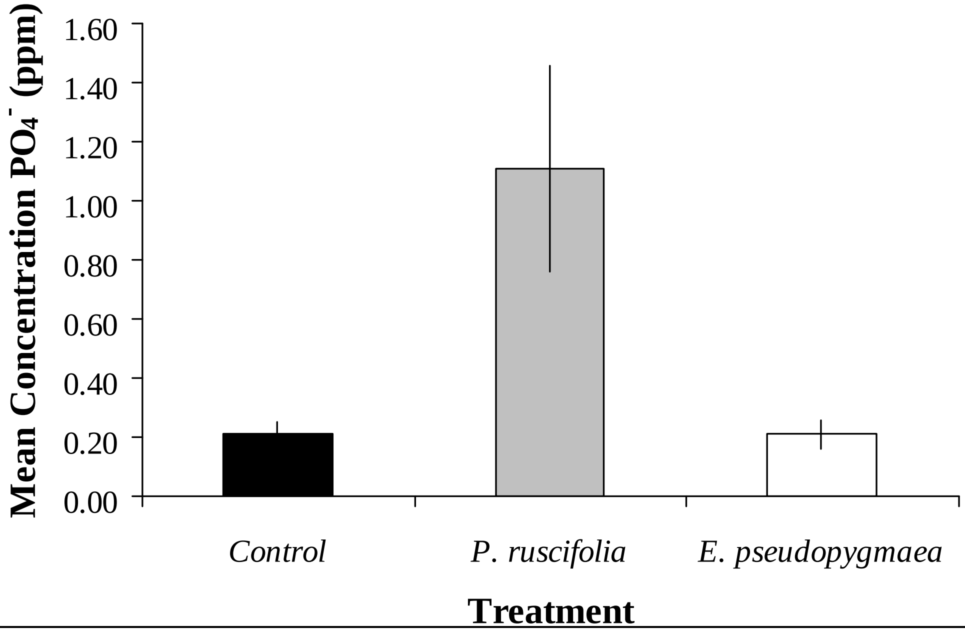

FIGURE 2. Mean concentration of PO_4^- for the different treatments (x ± SE) at the ridge site (Moss excluded). *P.ruscifolia* phosphate concentration significantly greater than pure mist (Kruskal-Wallis H = 6.56, p= 0.04; Tukey's test Q = 2.43, P = <0.05; N_C = 7 samples, $N_{P.r.}$ = 4 samples, $N_{E.p.}$ = 5 samples)

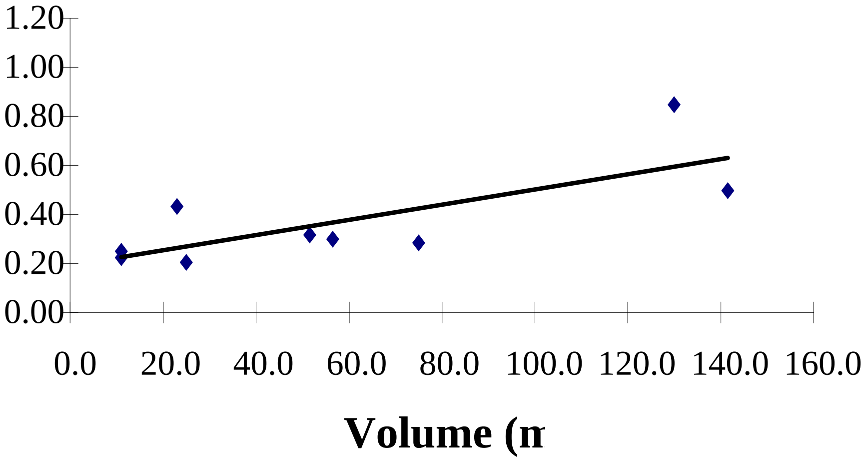

FIGURE 3. Simple linear regression plot relating volume of water collected in control to concentration of NO_3^- in control collector has a positive significant correlation. ($R^2 = 0.57$, $p = 0.02$, $N = 8$) As volume of water collected increases the concentration of NO_3^- also increases.

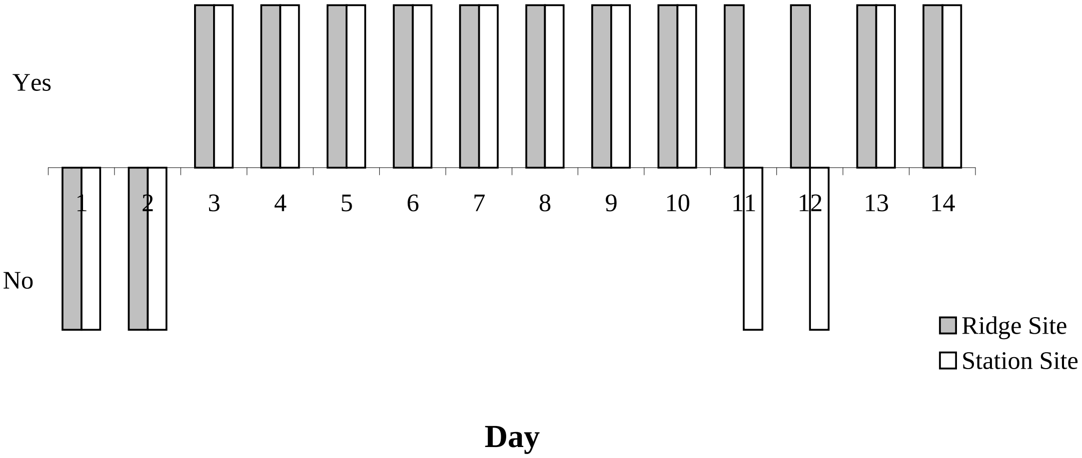

FIGURE 4. Comparison of days with mist precipitated at Station site (1520m) and Ridge site (1750m). There were two days where precipitation did not match between sites, indicating a difference in mist precipitation by altitude.