CHAPTER 4

RESULTS AND DISCUSSION

4.1 Greenhouse study

The different factors analyzed for the greenhouse results included: 1) two sites (uplands and lowlands), 2) two repeats (rainy season and dry season), 3) eight blocks (treatment replications) and 4) six treatments (Cont, PIF, BA1, CW, SC, and BA2). Site, repeat and treatment were evaluated as fixed factors since these were selected, while block was evaluated as a random factor due to variability of uncontrolled environmental conditions.

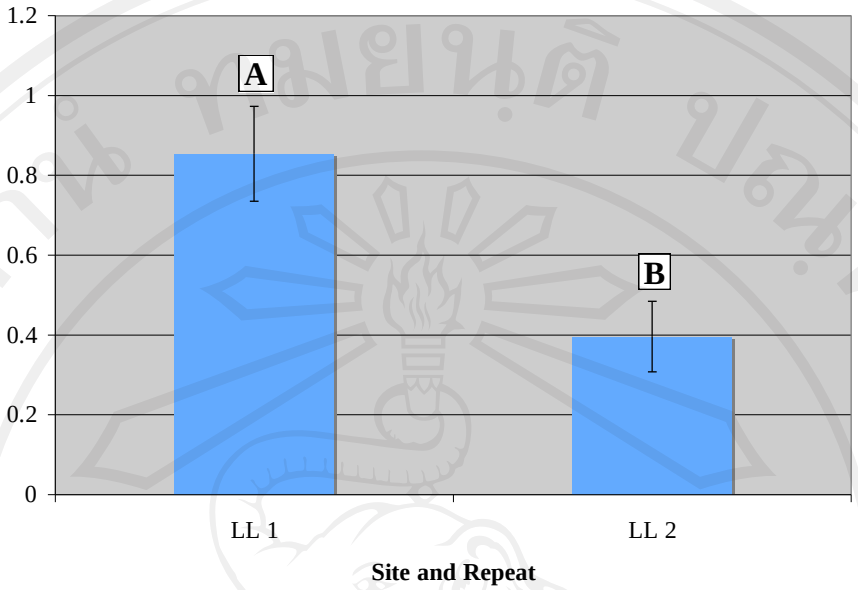
4.1.1 Number of plantlets

No significant difference in number of plantlets occurred between sites. However, season resulted in significant differences, and between treatments, there were significant differences.

Across treatments, rainy season produced a significantly ($P<0.05$) greater number of plantlets than the dry season (Fig. 10). This was consistent at both lowland and upland sites. There was no significant difference in numbers of plantlets between sites.

There are some significant differences in the number of emerged plantlets as a function of the interaction of treatment and repeat (Table 2 and Fig. 11): BA1 (rainy season) had a significantly ($P<0.05$) greater number of plantlets than PIF (rainy season), Cont, SC, BA2, CW, PIF, and BA1 (dry season) but not significantly higher than SC, BA2, CW and Cont (rainy season). SC (rainy season) had significantly ($P<0.05$) more plantlets than BA2, CW, PIF, and BA1 (dry season) but not

significantly more than BA2, CW, Cont, PIF (rainy season), Cont and SC (dry season). BA2, CW, and Cont (rainy season) had significantly ($P<0.05$) more than BA1 (dry season), but not significantly more then PIF (rainy season), Cont, SC, BA2, CW, and PIF (dry season). In conclusion, when looking at repeats individually (as a proxy for the rainy or dry season), in the rainy season, BA1 produced significantly ($P<0.05$) more plantlets than PIF, while in dry season, there was no significant difference. It is interesting to note that, while BA1 had the most number of plantlets emerge in rainy season it had the least number of plantlets emerge in dry season.



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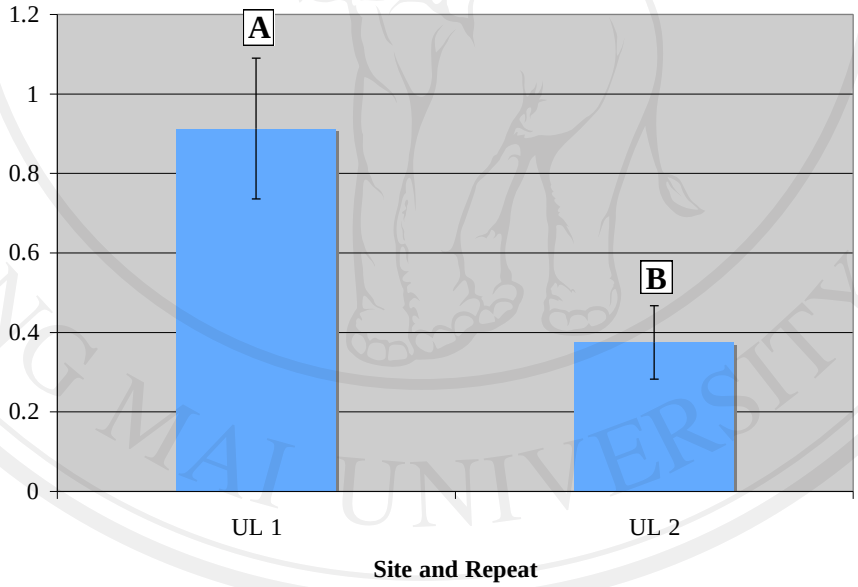


(b)

Figure 10: Average number of plantlets as a function of repeat, in the lowlands (a) and uplands (b). Mean separation, indicated with letters above bars, by Fisher's LSD, with alpha = 0.05, using PDMIX800. LSD = 0.294(a) and 0.299(b). Sites: lowlands (LL) and uplands (UL). Repeats: rainy season (1) and dry season (2). Vertical bars indicate standard error.

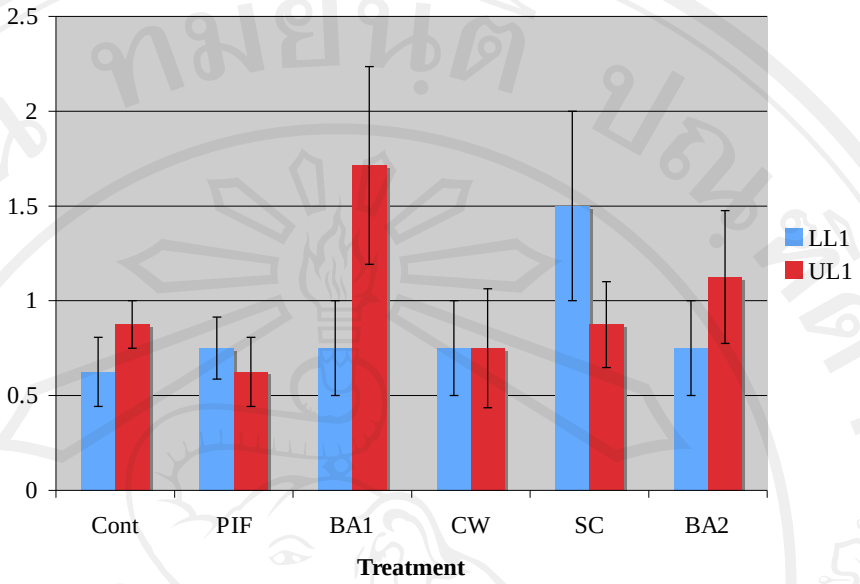
Table 2: Number of emerged plantlets as a function of the interaction of treatment and repeat.

Repeat[a]	Treatment[a]	Mean Number of Emerged Plantlets[b]	Standard Error	LSD
1	BA1	1.23 a	1.82	0.505
1	SC	1.14 ab	1.11	0.505
1	BA2	0.81 abc	0.85	0.505
1	CW	0.76 abc	0.77	0.505
1	Cont	0.76 abc	0.45	0.505
1	PIF	0.70 bcd	0.48	0.505
2	Cont	0.63 bcd	0.50	0.505
2	SC	0.63 bcd	0.81	0.505
2	BA2	0.44 cd	0.73	0.505
2	CW	0.38 cd	0.62	0.505
2	PIF	0.19 cd	0.54	0.505
2	BA1	0.07 d	0.25	0.505

[a]Repeats: rainy season (1) and dry season (2).

[b]Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2).

[c]Mean separation, indicated with letters, by Fisher's LSD, with alpha = 0.05, using PDMIX800



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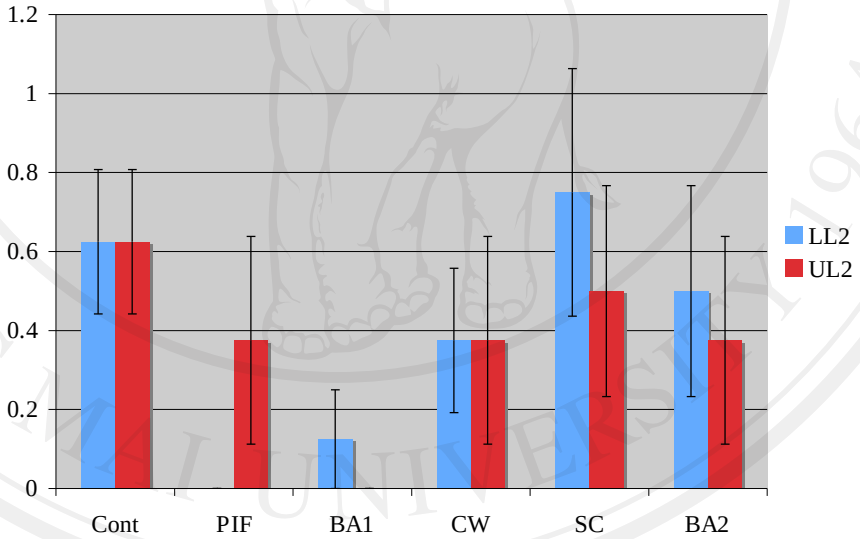


(b)

Figure 11: Average number of plantlets for lowlands and uplands in the rainy season (a) and dry season (b). Sites: lowlands (LL) and uplands (UL). Repeats: Rainy season (1) and dry season (2). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error; treatments were analyzed within sites.

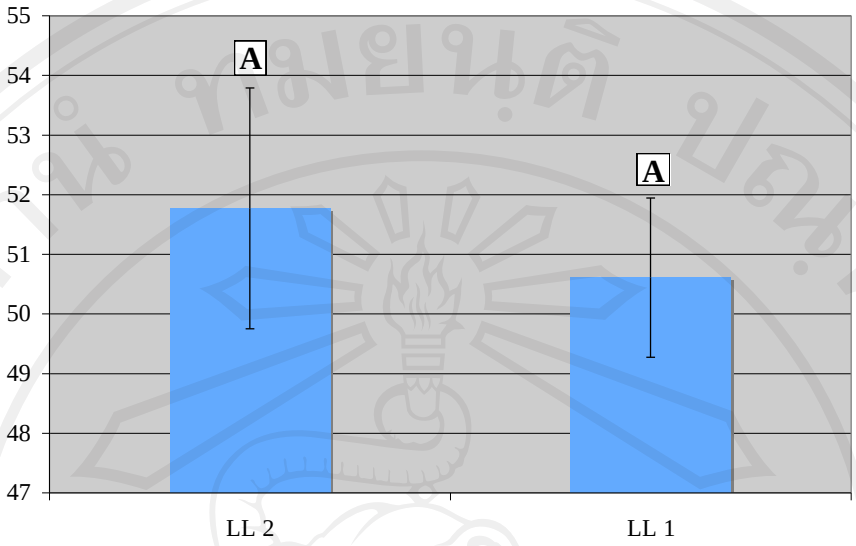
4.1.2 Time to plantlet emergence

Days to plantlet emergence was not significant between sites, but the timing of the repeats was significant by site. GDD to emergence was significant with respect to sites as well as repeats. There was no significant differences between treatments with regard to both days and GDD to plant emergence.

4.1.2.1 Cumulative days to plantlet emergence

There was not a significant difference between time to plantlet emergence between sites, averaging 50.79 days, but the timing of the repeats was significant by site. In the lowlands, plantlets in the rainy season emerged earlier than in the dry season, but it was not significant (Fig. 12a). However, for the uplands, plantlets in the rainy season emerged significantly ($P<0.05$) earlier than in the dry season (Fig. 12b). Across sites, plantlets in the rainy season emerged significantly ($P<0.05$) earlier than in the dry season (Fig. 14).

There were no significant differences between treatments for the time to plantlet emergence, although plantlets from all treated corms emerged later than the control during the rainy season (Fig. 13a) and most during the dry season (Fig. 13b).



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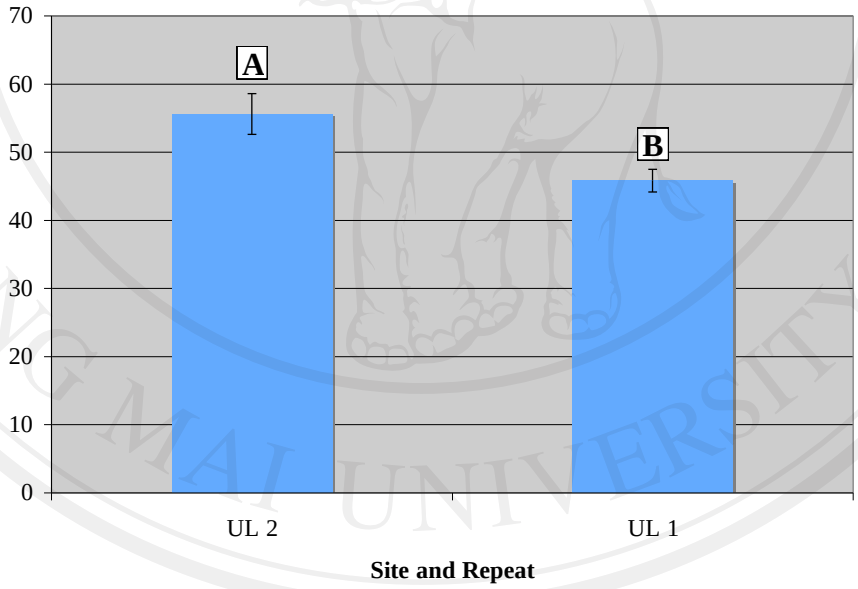
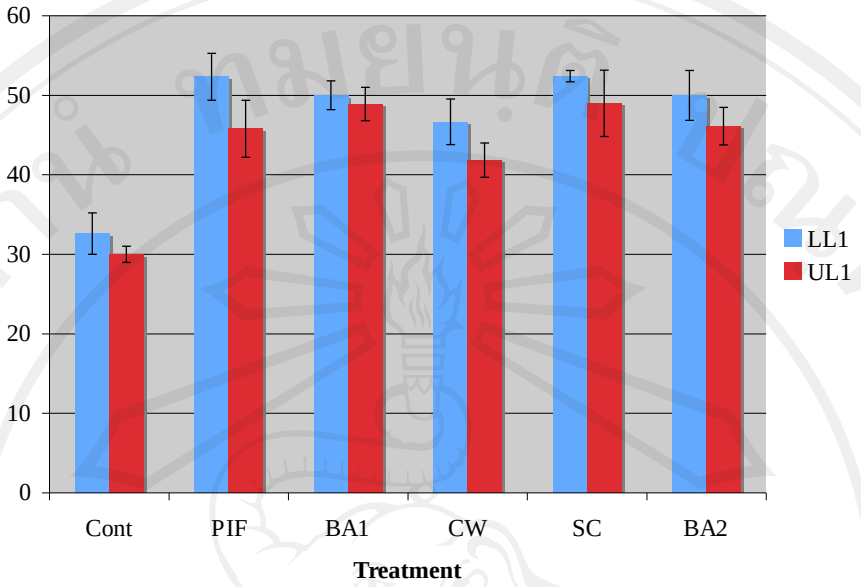


(b)

Figure 12: Average cumulative days to plantlet emergence as a function of repeat, for lowlands site (a) and uplands site (b). Mean separation, indicated with letters above bars, by Fisher's LSD, with alpha = 0.05, using PDMIX800. LSD = 4.983(a) and 7.001(b). Sites: lowlands (LL) and uplands (UL). Repeats: rainy season (1) and dry season (2). Vertical bars indicate standard error.



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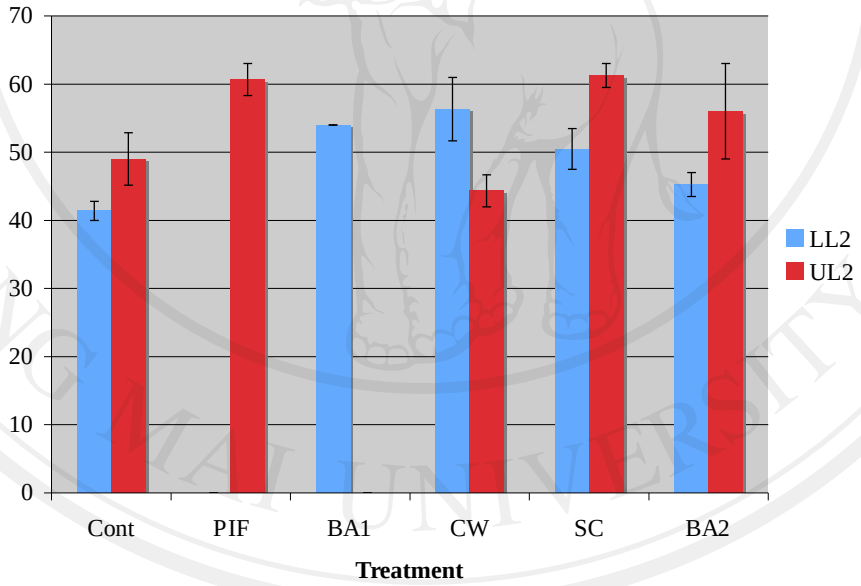


(b)

Figure 13: Average cumulative days to emergence for lowlands and uplands in the rainy season (a) and dry season (b). Sites: lowlands (LL) and uplands (UL). Repeat: rainy season (1) and dry season (2). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error; treatments were analyzed within sites.

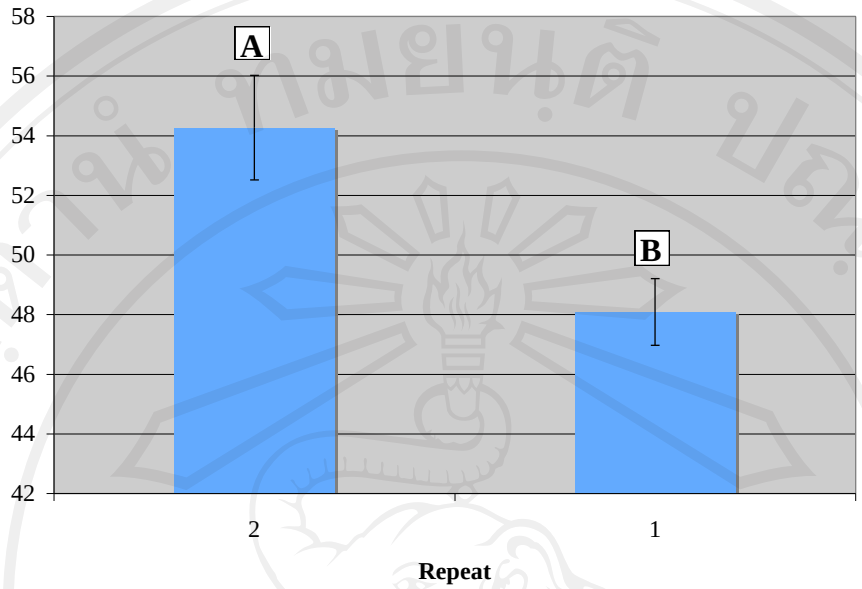
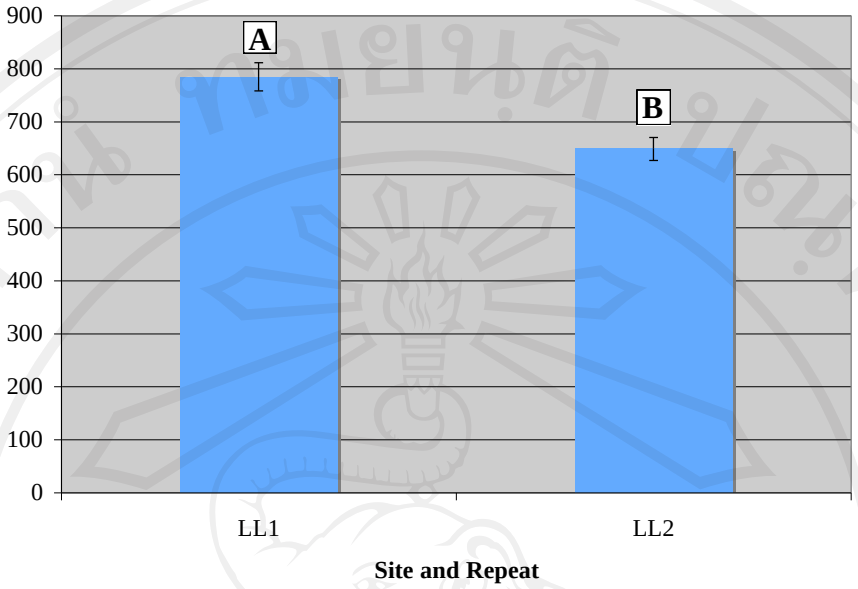


Figure 14: Average cumulative days to emergence as a function of repeat, across sites and treatments. Mean separation, indicated with letters above bars, by Fisher's LSD, with alpha = 0.05, using PDMIX800. LSD = 5.39. Repeats: rainy season (1) and dry season (2). Vertical bars indicate standard error.

4.1.2.2 Growing degree days to plantlet emergence

Across repeats, plantlets in the uplands emerged with significantly ($P<0.05$) fewer GDD's than in the lowlands (LL > UL) (Fig. 16a), while simultaneously, plantlets emerged with significantly ($P<0.05$) fewer accumulated GDD's earlier in the dry season than in the rainy season (repeat 1 > repeat 2) (Fig. 16b). In both sites, plantlets in the dry season emerged with significantly ($P<0.05$) fewer accumulated GDD's than in the rainy season (Fig. 15ab). There were no differences in GDD's between treatments, although plantlets from all treated corms emerged later than the control during the rainy season (Fig. 17a) and most during the dry season (Fig. 17b).



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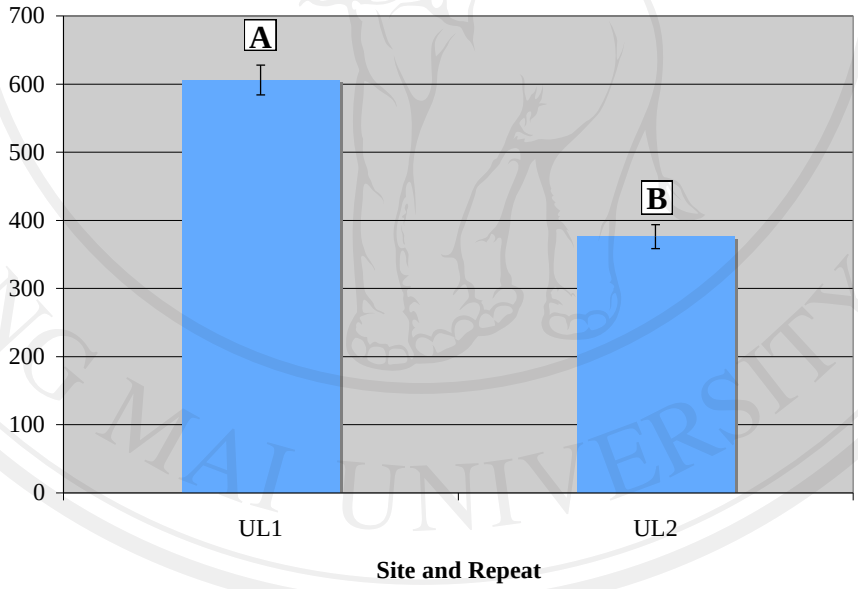
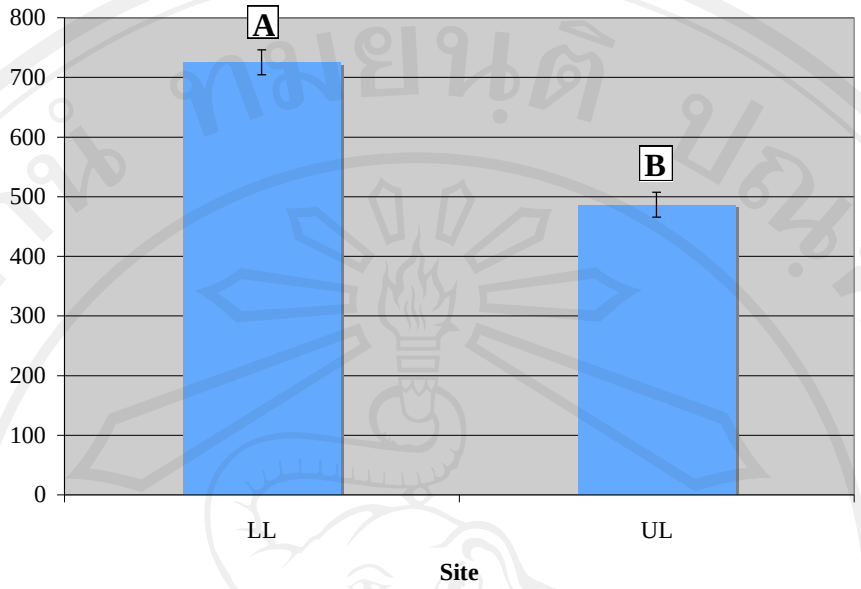


(b)

Figure 15: GDD to emergence as a function of repeat, for lowlands site (a) and uplands site (b). Mean separation, indicated with letters above bars, by Fisher's LSD, with alpha = 0.05, using PDMIX800. LSD = 70.396(a) and 81.039(b). Sites: lowlands (LL) and uplands (UL). Repeats: rainy season (1) and dry season (2). Vertical bars indicate standard error.



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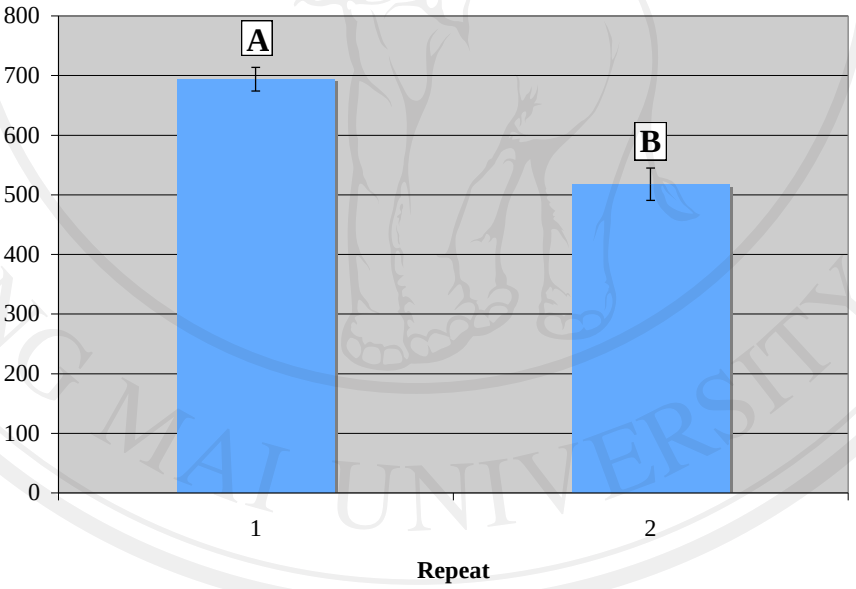
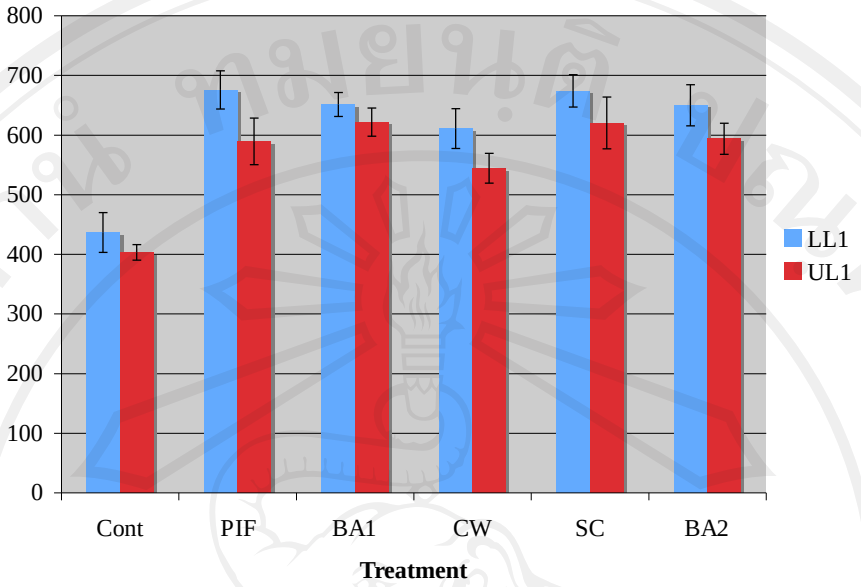


(b)

Figure 16: GDD to emergence as a function of site (a) and repeat (b). Mean separation, indicated with letters above bars, by Fisher's LSD, with alpha = 0.05, using PDMIX800. LSD = 63.263(a) and 69.545(b). Sites: lowlands (LL) and uplands (UL). Repeats: rainy season (1) and dry season (2). Vertical bars indicate standard error.



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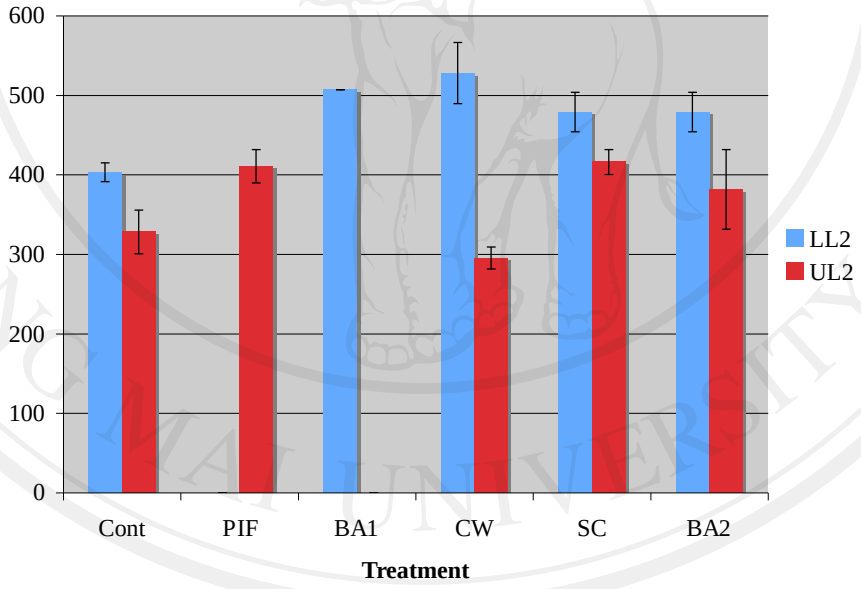


(b)

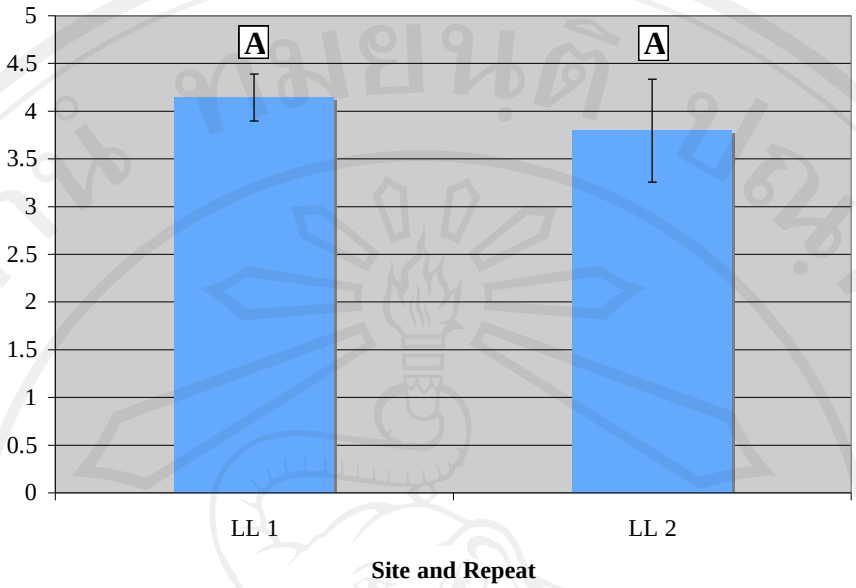
Figure 17: Average GDD to emergence for lowlands and uplands in the rainy season (a) and dry season (b). Sites: lowlands (LL) and uplands (UL). Repeat: dry season (2). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error; treatments were analyzed within sites.

4.1.3 Mean final circumference of plantlets

Site and repeat had no significant difference, with respect to mean final circumference, but some significant difference occurred between treatments.

Differences were not significant when running circumference as a function of site and repeat, although there was a slightly higher mean circumference across treatments in the lowlands and the rainy season (LL1 > LL2 and UL1 > UL2) (Fig. 18).

Between treatments, during the rainy season, the control treatment had higher mean circumferences than other treatments, and the SC treatment had lower mean circumferences than Cont, PIF, BA1, CW and BA2 (Fig. 19a). In the dry season, control treatment had higher mean circumferences than other treatments, with the exception of lowlands BA1, and the SC treatment had lower mean circumferences than other treatments (Fig. 19b). BA1 had a high mean circumference in the lowlands, but did not have any plantlets in the uplands to compare. Altogether, across sites and repeats, removing the control treatment, there was no significant differences in circumference between treatments with BA1>BA2>CW>PIF>SC, with the exception of SC which had a significantly ($P<0.05$) lower mean circumference than BA1, BA2, CW and PIF (Fig. 20).



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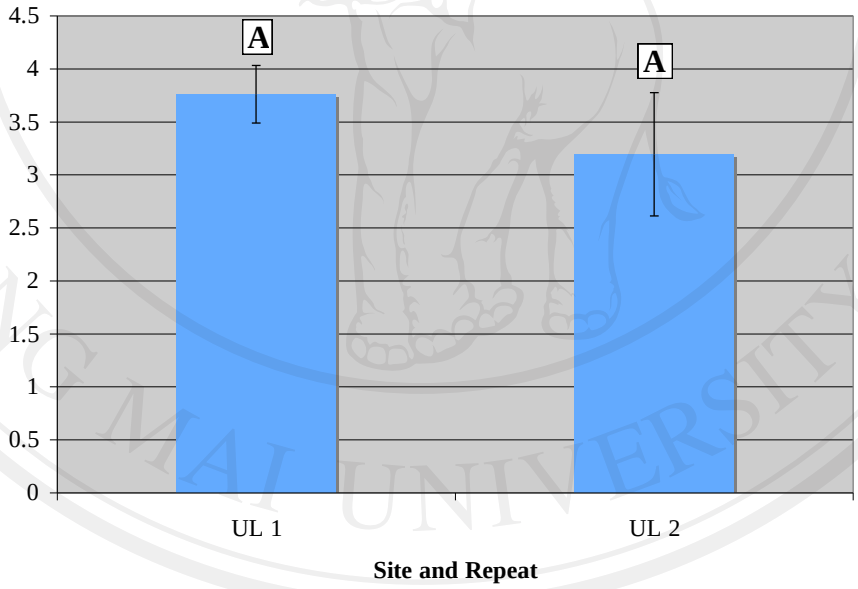
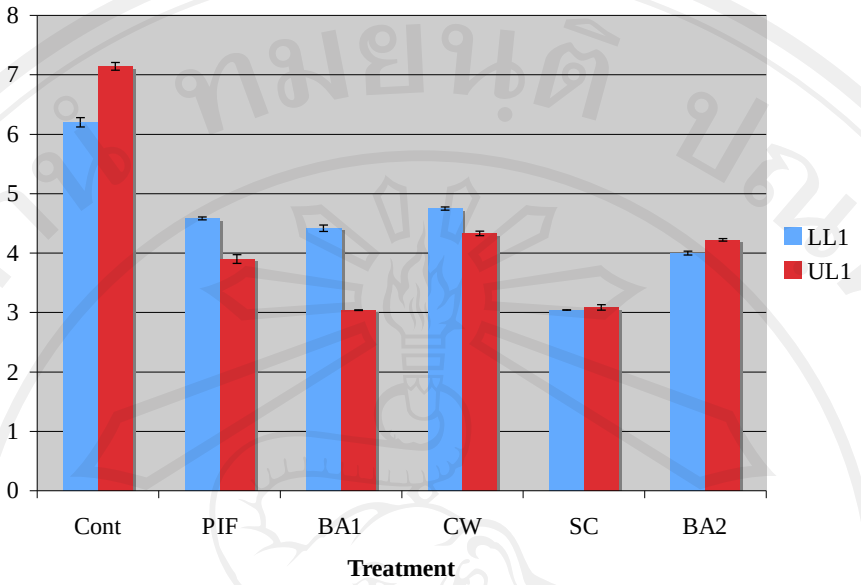


(b)

Figure 18: Circumference as a function of repeat, for lowlands site (a) and uplands site (b). Mean separation, indicated with letters above bars, by Fisher's LSD, with alpha = 0.05, using PDMIX800. LSD = 1.042(a) and 1.199(b). Sites: lowlands (LL) and uplands (UL). Repeats: rainy season (1) and dry season (2). Vertical bars indicate standard error.



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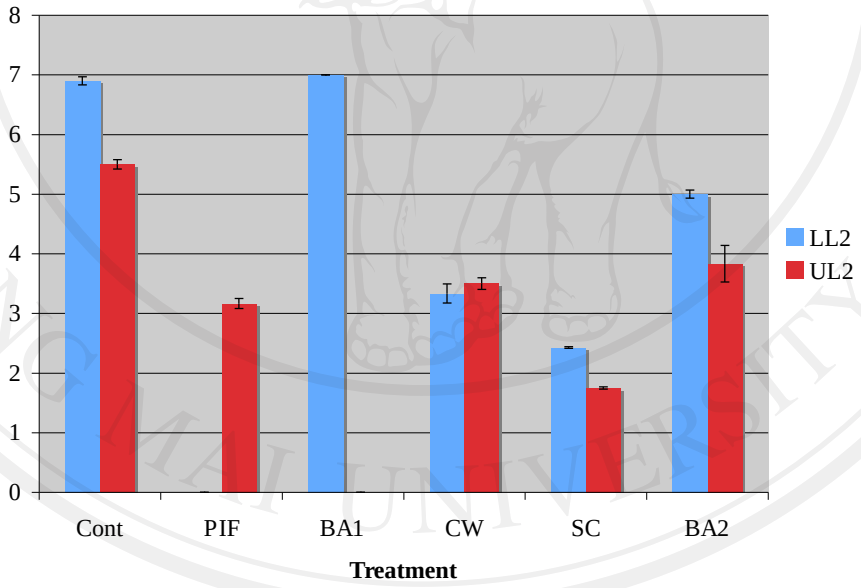


(b)

Figure 19: Mean final circumference of plantlets for lowlands and uplands in rainy season (a) and dry season (b). Sites: lowlands (LL) and uplands (UL). Repeat: rainy season (1) and dry season (2). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error; treatments were analyzed within sites.

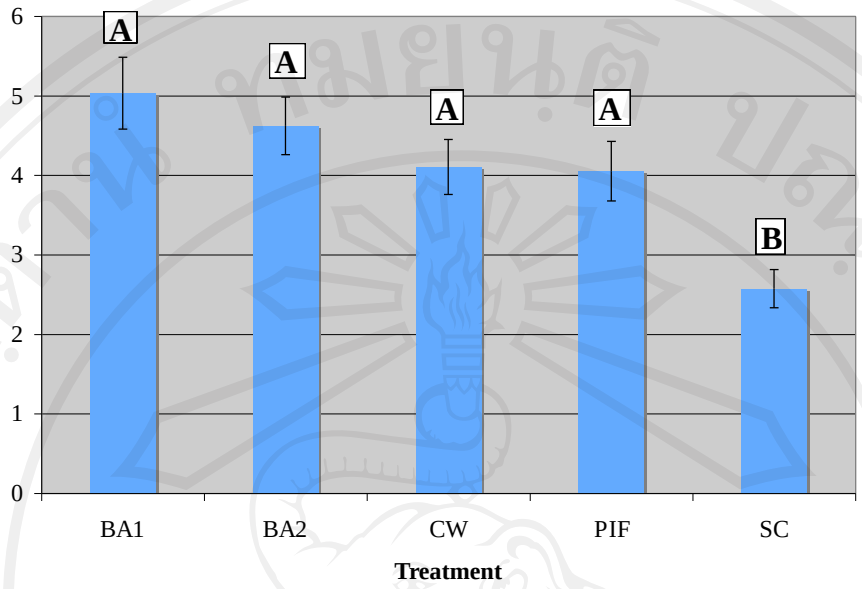


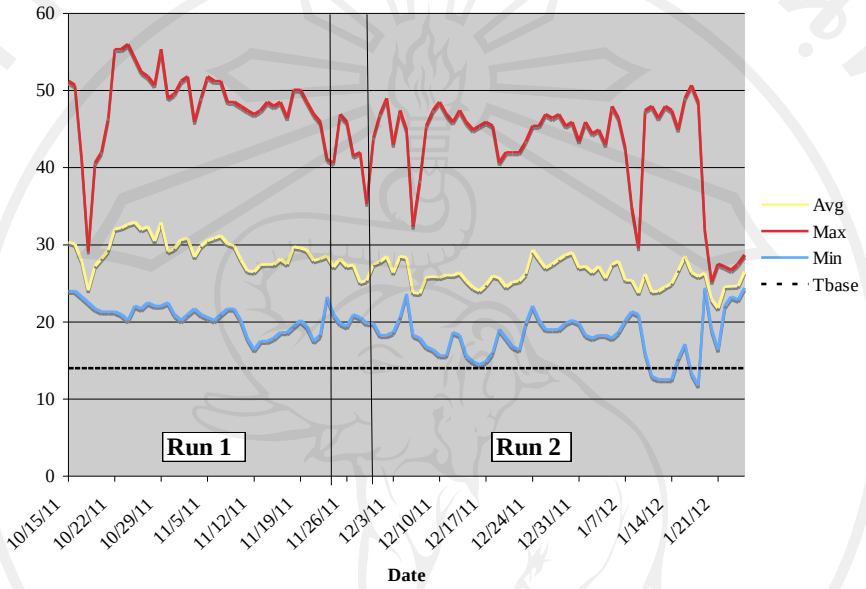
Figure 20: Circumference as a function of treatment. Mean separation, indicated with letters above bars, by Fisher's LSD, with alpha = 0.05, using PDMIX800. LSD = 1.371. Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error.

4.1.4 Other factors

4.1.4.1 Temperature in greenhouse bed

There were much higher maximum temperatures in the greenhouse beds than the ambient maximum, with slightly higher temperature readings in the lowland beds. Both sites had maximum temperatures above the optimal range for banana plant growth. Minimum temperatures were relatively the same between the greenhouse beds and ambient temperatures, with few readings below the base temperature for banana (Fig. 21). Although the data loggers were not used during the full length of

time of the study, it was evidenced that temperatures decreased as the season changed from warm to cold (starting around mid-November).



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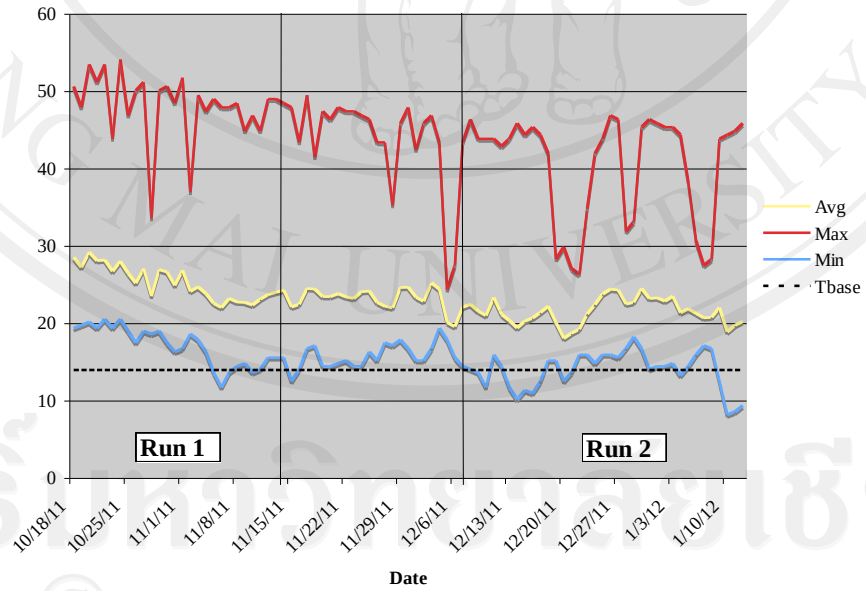

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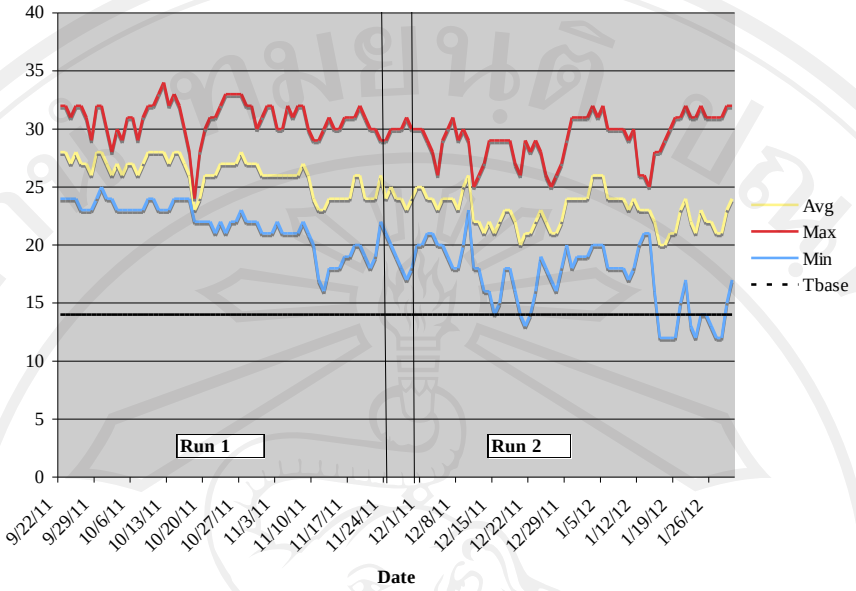

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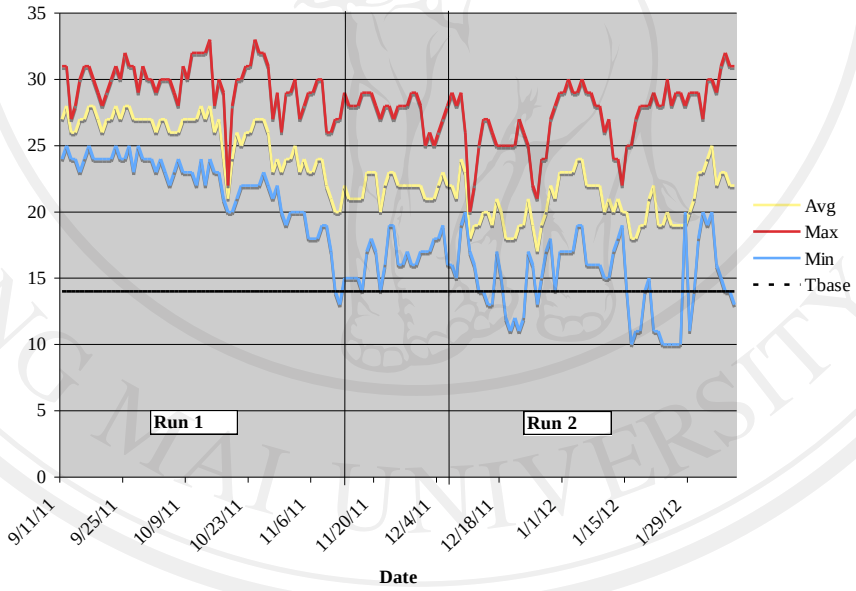


(d)

Figure 21: Temperature measurements for lowlands (a) and uplands (b) greenhouse planting beds (taken from HOBO® Data Logger) and lowlands (c) and uplands (d) ambient temperatures (taken from Wunderground, 2012) with average, maximum, minimum daily temperatures and base temperature (14 ºC) for bananas. Repeats: rainy season (1) and dry season (2).

4.1.4.2 Pests and diseases

There were several signs of pest and disease presence in the corms obtained for greenhouse studies. These occurred more in corms gathered in the lowlands than in the uplands. The three main problems found were: weevil damage from the *Cosmopolites sordidus* Germar adult and larva, nematode damage, and fungus damage (Fig. 22). Weevil damage was evident in the extensive tunneling, that sometimes led to decay in the tunnel area. Nematodes were evident in a reddish color surrounding the root area after the cortex was removed. Fungal damage was marked by soft, grayish cortex in the area where corm was damaged from removal from the mother plant.

(a)  (b) 

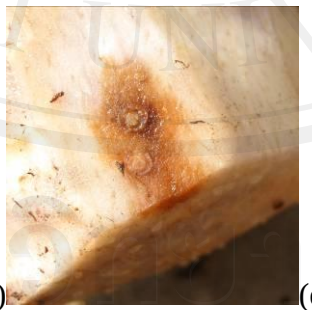
(c)  (d)  (e) 

Figure 22: Pest and disease problems in the greenhouse experiment: *Cosmopolites sordidus* Germar in adult (a) and larval stages (b), caused significant damage to some corms (c). Nematode evidence was also found (d) as were some fungal problems (e).

There were also reddish and yellow flecks in the central cylinder of the some corms, which might suggest the possibility of Fusarium wilt infection. All pest and disease damage was removed with aseptic knives before planting. No pest or disease presence was observed after planting.

4.1.5 Greenhouse discussion

In summary, the timing of repeat significantly influenced the number of produced plantlets, cumulative days to emergence, cumulative GDD to emergence, but not circumference of plantlet corms. Site only significantly influenced cumulative GDD to emergence. Treatment and repeat significantly influenced the number of emerged plantlets; during the rainy season BA1 produced significantly more plantlets than PIF, while during the dry season, there was no significant difference by treatment. For the dependent variable of circumference, only SC produced significantly smaller plantlets than the other treatments.

Benzylaminopurine is a growth regulator and is known to reduce the apical meristem dominance and induce the formation of axillary and adventitious shoots (Jafari, 2011). Although PIF treatment destroyed the apical meristem, reducing auxin production, and thus apical dominance (Boss, 2008), it did not effect levels of cytokinins, inducing axillary growth, thereby explaining why the BA1 treatment would produce more plantlets than the PIF treatment.

The SC treatments produced smaller corm circumferences than the other treatments, which was likely the result of the splitting of the corm into four sections. A study done by Ojeifo (2006), evaluating split seeds of *Telfairia occidentalis* for production, found that the girth of vines developed from whole seeds was comparable to those halved and quartered, but greater than the 1/8 seed, concluding that food

reserves were higher for the larger sections of seed. It is possible that,for banana corms, with smaller sections, there are fewer resources available for the plant to draw from. Further study should be conducted on the effects of split-corm on produced plantlets in bananas.

The numbers of plantlets produced from treated corms were significantly lower than the numbers given in the literature (Boss, 2008; Osei, 2005; Dzomeku, 2000). A possible reason is that these studies regenerated plantlets to multiple generations, adding up the cumulative number of plantlets possible. Our study only used one generation of plantlets. Preece (2008) mentions, "shoot explants from juvenile plants generally proliferate more axillary shoots than shoot explants from adult forms." Perhaps treating the plantlets produced from our treatments would produce increased numbers of plantlets.

Another possible reason for lower numbers of plantlets than expected from the literature could be a result of the cultivar used. When working with tissue culture, cultivar has an impact on how many axillary buds develop, with diploids producing more buds than most commercial cultivars, which are typically triploids (Singh, 2011). In order to better understand the role the 'Kluai nam wa' cultivar played in numbers of plantlets produced, a study comparing different cultivars would need to be undertaken.

As previously mentioned, existing literature states that temperature plays an important role in the growth of bananas with 22-31 ºC being the ideal temperatures for optimal growth in bananas (Robinson, 2010). It is likely that temperature was one of the factors influencing the number of plantlets produced between both sites as well as repeats. Temperature decrease as the season changed from rainy season to dry

season with slightly higher temperature in the lowlands than in the uplands. Measured low temperatures were very close to ambient lows and included some periods of time, during the evenings, below the base growing point for bananas. High temperatures were also often above the optimal temperature for banana plant growth, 37 ºC (Robinson, 2010). Cumulative GDD to emergence also evidenced there was greater accumulation of heat at the lowlands site and during the first repeat. Perhaps if there had been a better regulation of temperatures, maintaining temperatures between the optimal range for bananas, results would have been more consistent across sites and repeats. Regulation of greenhouse temperatures would allow farmers in uplands to compete on equal levels with lowland farmers in macropropagation.

It is also possible that initial corm size played an important role in determining how effective the treatments were. Since axillary buds are formed from meristems in the axis of leaves (Preece, 2008), sucker production is controlled by the number of viable axillary buds on the corm prior to treatment (Ross, 1992). Osei's (2005) research found that small suckers below 25 cm tall did not respond to treatments as well as those between heights of 35-100 cm because of the number of preformed viable axillary buds prior to treatment. Although it was not possible to evaluate this in our study, because there were not adequate numbers of large and small corms used, more research could be done to better understand this possible correlation.

Although it is unknown how pests and diseases affected our results, they likely had some effect. In tissue culture research, axillary shoot production and thus, the number of established plants, has a tendency to be lower in disease infected cultures than in healthy ones (Preece, 2008). Fungal diseases, such as Fusarium wilt, were a problem, especially in the lowlands, where the temperature is higher. This is as

expected, as fungus thrives in moist, warm environments (Nelson, 2006). Although the greenhouse bed was treated with a fungicide, fungus had already affected the corms before planting. Perhaps it would have been better to have dipped the corms in fungicide directly after digging them up to prevent any fungal spread in damaged areas. Without having to pare back as much cortex to clean the corms, this would also result in larger corms used for treatments. With regard to pests and diseases, it is important for farmers to start with clean planting material and a clean field if they plan to practice macropropagation on a large scale (Robinson, 2010). Although more expensive, it would be best if these farmers started their field with tissue cultured bananas to ensure clean, healthy material for propagation.

Plantlets were transplanted after three leaves were present. It is not known when the ideal stage for plantlet transplanting occurs and whether more plantlets might emerge with earlier excising. Boss (2008) recommends excising PIF plantlets below 1.5 cm in diameter with 2-5 leaves while those greater than 1.5 cm were re-treated while still attached to the corm. This enabled greater production of plantlets. More research is needed to determine transplanting best practices.

BA1 treatment produced the greatest number of plantlets in the first repeat, but when running an economic analysis to see if BA is something that could realistically be used by low-income farmers, BA1 does not appear promising. Taking into consideration the cost of purchasing the chemical, even at the discount price received through Hohenheim University (13,223 THB for 25g BA, using 2.25g and 1.126g for BA1 and BA2, respectively, per treatment), and an average of the total number of plantlets produced during the first repeat (12.5 plantlets), plants would have to be sold for 95 baht per plant to break even. This is without taking into account cost of water,

greenhouse beds, soil for transplanting, or pots. This is unrealistic since the current market rate is 5 baht per plant. Although plants guaranteed to be pest and disease free should allow prices to increase somewhat, it would be difficult to find buyers at this steep price. Although the BA2 treatment produced fewer plantlets, it had a better return. When using the same information with an average of the total number of plantlets produced in the first repeat (7.5), plants would have to be sold for 76 baht per plant to break even. Again, this is unrealistic, but slightly closer to market value.

That being said, there still might be hope for the use of BA in macropropagation of bananas. There are a number of factors for which further research is need. The ideal concentration of BA for optimizing number of plantlets at lowest costs is unknown, because there is as no literature that utilized dipping banana corms in BA concentrations. It is also unknown how many corms can be dipped in one solution of BA without losing effectiveness. These factors, in combination with other recommendations made, could result in significantly higher production possibilities.

However, it might be more beneficial to pursue the lower cost treatments, fine-tuning them in combination with the other recommendations made or perhaps combining mechanical injury with locally available growth regulators. Results indicated that SC produced more plantlets (an average of 1.14 plantlets) than other treatments besides BA1 (an average of 1.23 plantlets). This is perhaps a better low-input treatment to target, especially for farmers with little money for start-up costs. There are also other promising methods of macropropagation and cultivars that should be included in future studies (Simmonds, 1966; Faturoti, 2002; Baiyeri, 2005; Pillay, 2011).

Finally, more time was needed for data collection in the greenhouse study to better analyze the effect of the different treatments. Although in the literature, plantlets emerged and data was collected at 6-8 weeks (Boss, 2008) and 3 weeks (Osei, 2005), in existing literature, in our study, plantlets were still emerging at 60 days. This was especially evident during the dry season, when plantlets emerged later. Perhaps different results would have emerged if we had run the experiment a few more weeks.

Recommendations:

- Greenhouse temperatures need to be better regulated to achieve steady temperatures during the day and at night.
- It is important to start with clean, healthy planting material when setting up a field for commercial propagation.
- Fungicide should be used on corms directly after digging up before fungus has the chance to spread to damaged areas.
- Data should be collected for a longer period of time; 60 days was not long enough to determine numbers of plantlets for each treatment during the time of year when this experiment was conducted (perhaps a different experimental schedule would have produced plantlets more quickly).

Further research is needed:

- to compare ‘Kluai nam wa’ to other cultivars
- to determine whether smaller circumference size of SC treated corms affects the plants later in life,
- to determine if there are increased numbers of plantlets when using second

generation corms,

- to determine if a correlation exists between starting corm size and effectiveness of treatments,
- to fine-tune BA concentrations for optimal levels of plantlet production and determine how many corms can effectively be used in one solution preparation, and
- to see if there is increased effectiveness for lower cost treatments with better regulated temperatures and healthy corms.

Although these techniques did not produce the numbers of plantlets expected from existing literature, it is important for research to continue. It is especially important to seek to understand how to help farmers multiply corms with lower input costs than setting up a tissue culture lab and how to produce greater returns than direct transplanting of existing pups. Answers to these questions will help to contribute enough planting stock for upland farmers to produce fruit and provide fodder for their pigs, enabling them to participate in local markets, and bettering their lives.

4.2 Field study

The different factors analyzed included: 1) two sites (uplands and lowlands), 2) six blocks (treatment replications) 4) five treatments (Cont, MM, BA1, BA2, and CW). Site and treatment were analyzed as fixed effects since these were selected by the experimenters, while block was analyzed as a random variable due to variability of uncontrolled environmental conditions.

4.2.1 Number of emerged plantlets

No significant differences occurred in the ratio of emerged plantlets between uplands and lowlands as well as between treatments.

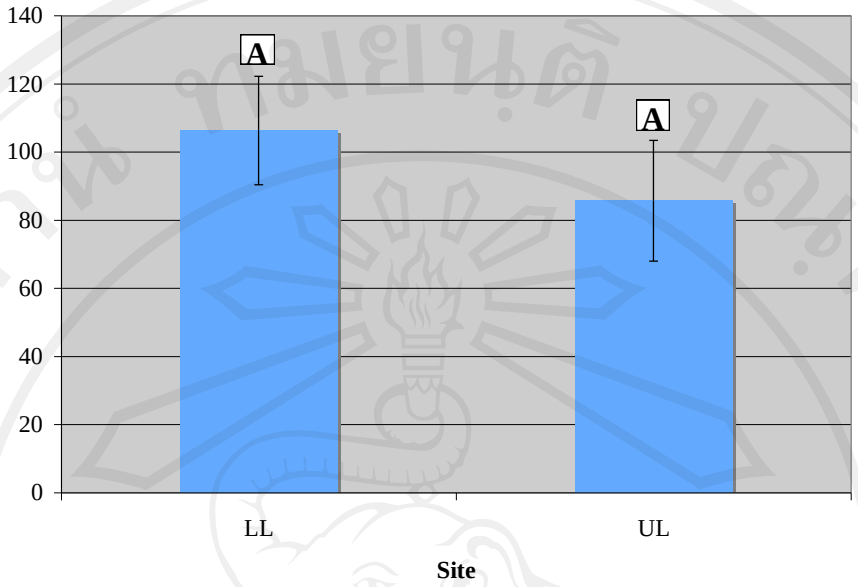
The ratio of emerged plantlets as a function of site was not significantly different between the uplands and the lowlands although more plantlets were produced in the lowlands than in the uplands (Fig. 23a). In terms of total number of plantlets produced, treatments BA1 and CW produced more than the other treatments while MM produced the fewest number of plantlets within sites (Fig. 24). However, for the ratio of emerged plantlets as a function of treatment BA1 > MM > CW > BA2 > Cont although it was not significantly different between treatments (Fig. 23b).

4.2.2 Time to plantlet emergence

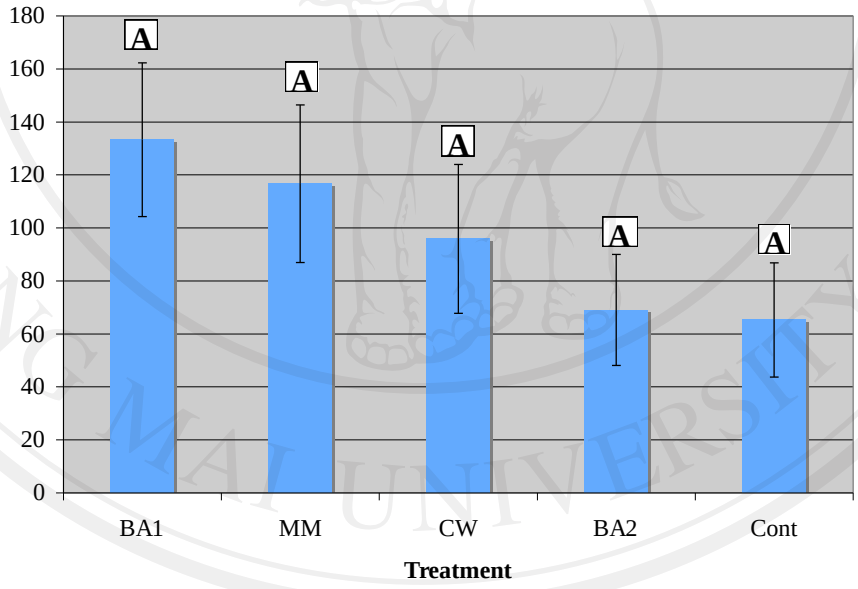
There was no significant difference in time of plantlet emergence, both in terms of cumulative days as well as GDD's, between sites or treatments.

4.2.2.1 Cumulative days to emergence

Plantlets in the uplands emerged slightly earlier than those in the lowlands, but not significantly different (Fig. 25a). With respect to treatment, Cont > BA1 > BA2 > CW > MM, but again, there were no significant differences (Fig. 25b).



(a)



(b)

Figure 23: Ratio of emerged plantlets as a function of site (a) and treatment (b). Mean separation, indicated with letters above bars, by Fisher's LSD, with alpha = 0.05, using PDMIX800. LSD = 64.248(a) and 72.818(b). Sites: lowlands (LL) and uplands (UL). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error.

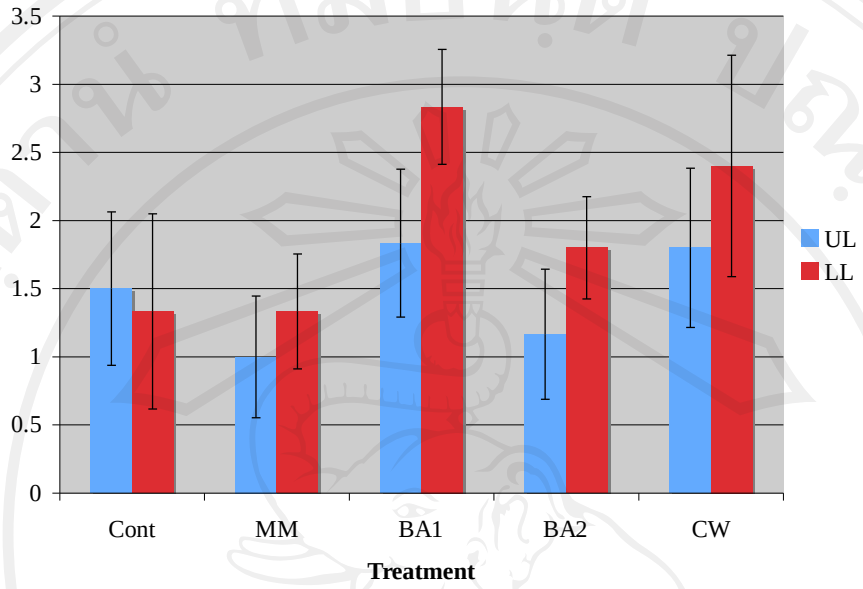
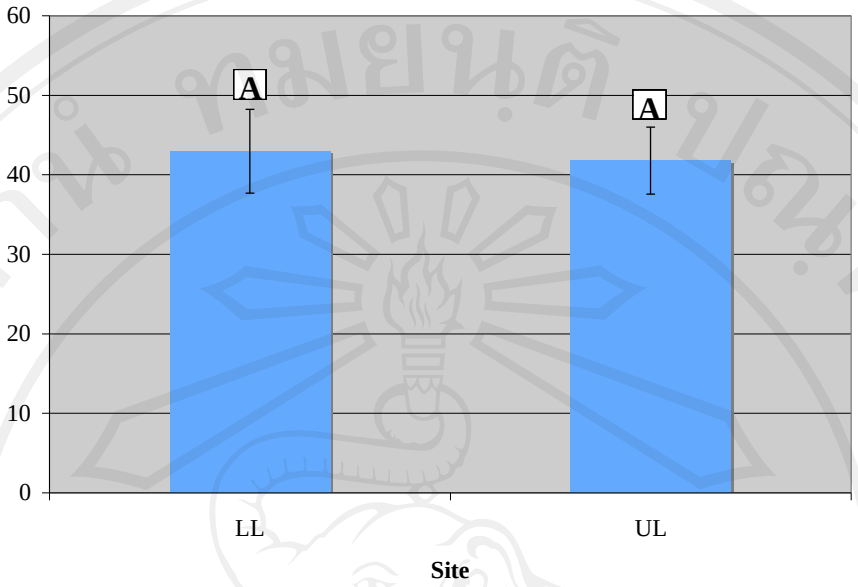
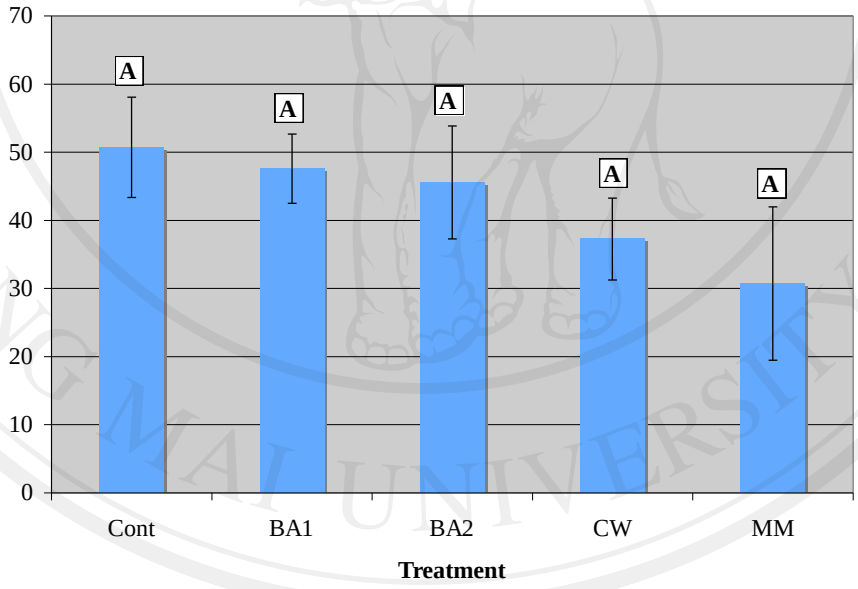


Figure 24: Average number of plantlets across sites and treatments. Sites: uplands (UL) and lowlands (LL). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error; treatments were analyzed within sites.



(a)



(b)

Figure 25: Mean cumulative days to emergence as a function of site (a) and treatment (b). Mean separation, indicated with letters above bars, by Fisher's LSD, with alpha = 0.05, using PDMIX800. LSD = 14.586(a) and 23.037(b). Sites: lowlands (LL) and uplands (UL). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error.

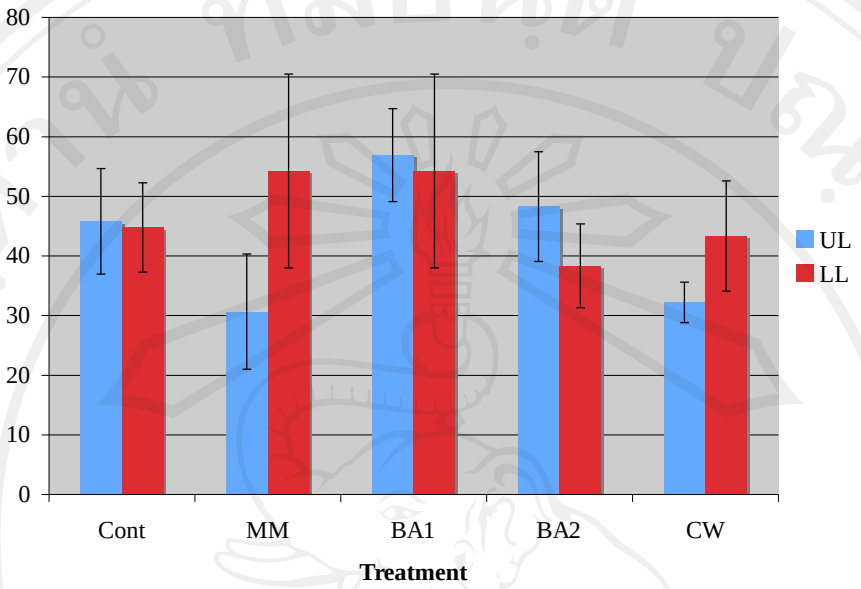
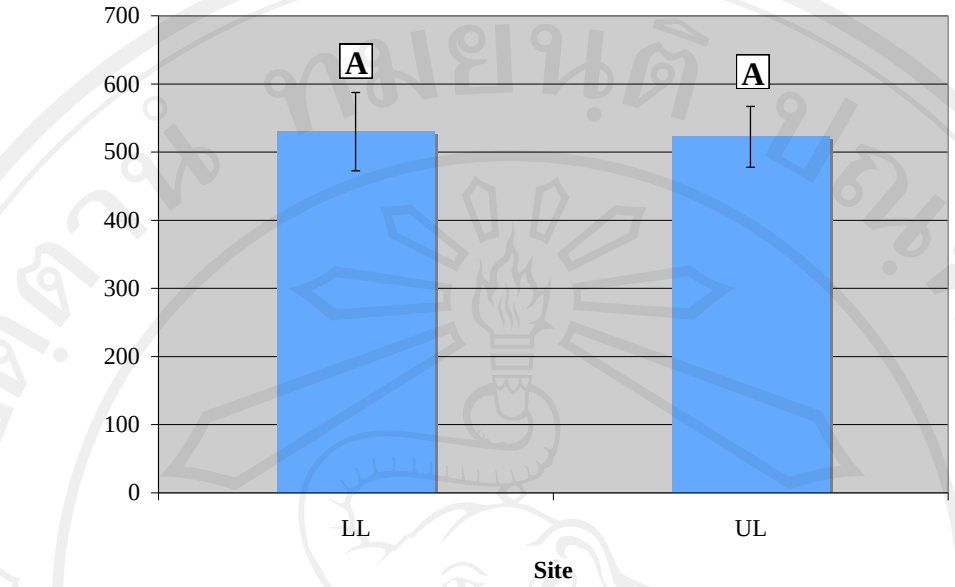


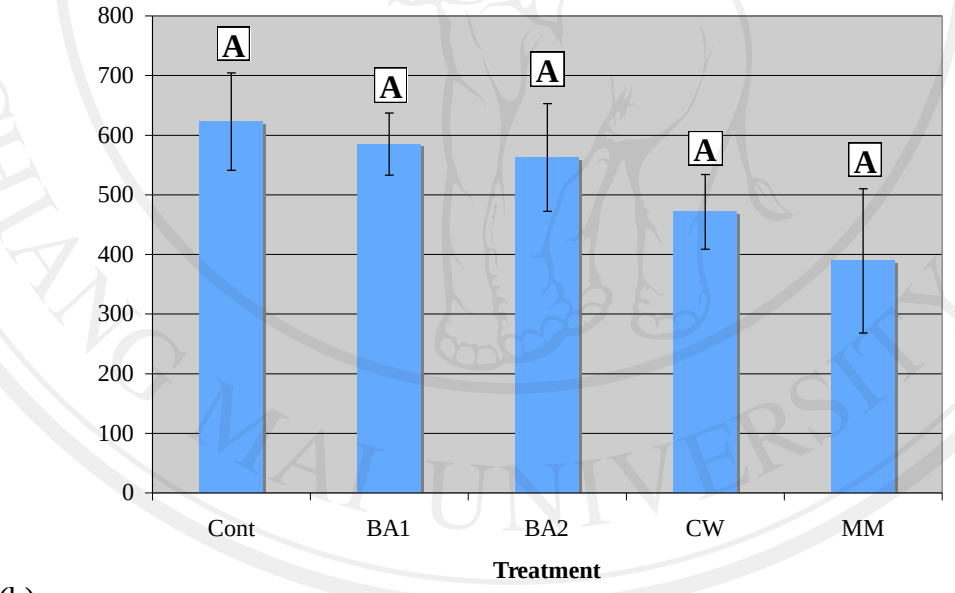
Figure 26: Average cumulative days to emergence between sites and treatments. Sites: uplands (UL) and lowlands (LL). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error; treatments were analyzed within sites.

4.2.2.2 Growing degree days to emergence

Plants in the uplands emerged with slightly fewer accumulated GDD's than those in the lowlands, but not significantly (Fig. 27a). A similar trend emerged for the effects of treatment on cumulative GDD as for cumulative days to emergence: Cont > BA1 > BA2 > CW > MM, but again, there were no significant differences (Fig. 27b).



(a)



(b)

Figure 27: Mean GDD to emergence as a function of site (a) and treatment (b). Mean separation, indicated with letters above bars, by Fisher's LSD, with alpha = 0.05, using PDMIX800. LSD = 156.846(a) and 247.723(b). Sites: lowlands (LL) and uplands (UL). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error.

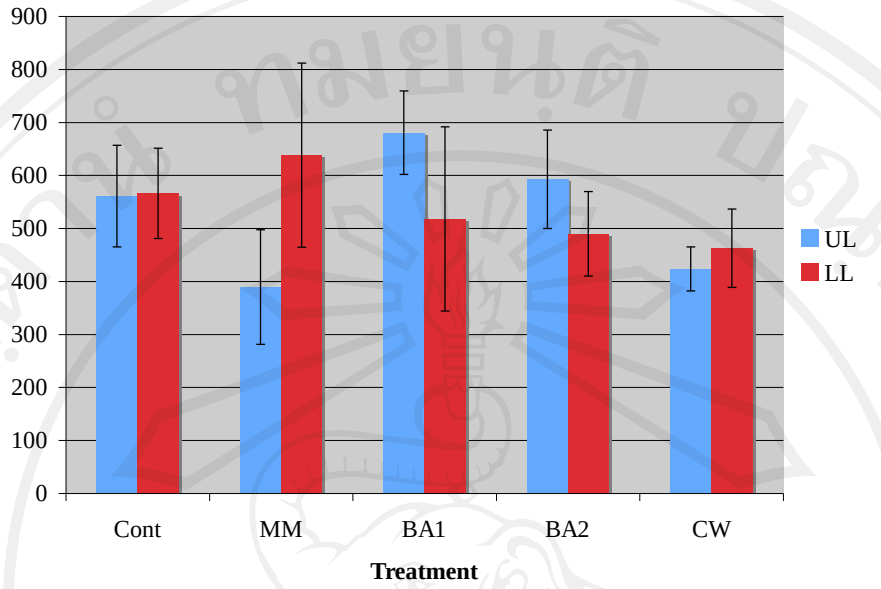
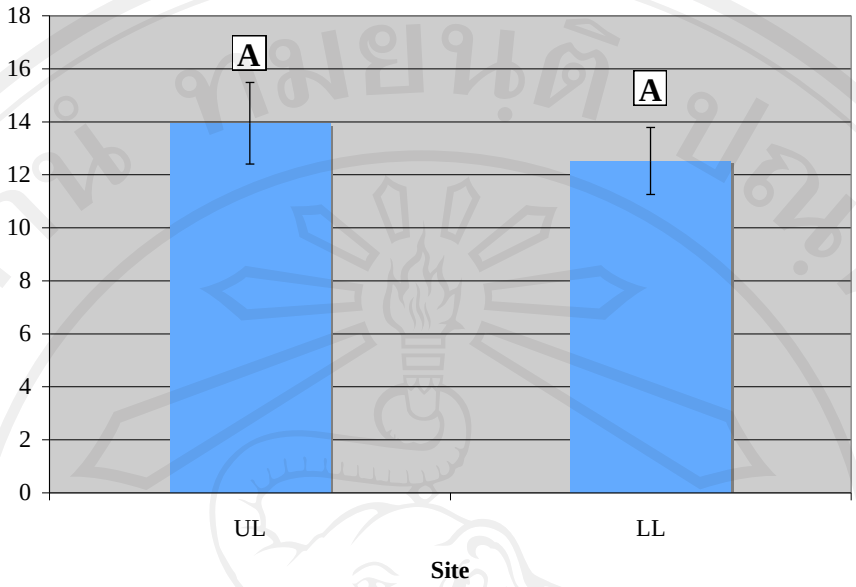


Figure 28: Average GDD to emergence between sites and treatments. Sites: uplands (UL) and lowlands (LL). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error; treatments were analyzed within sites.

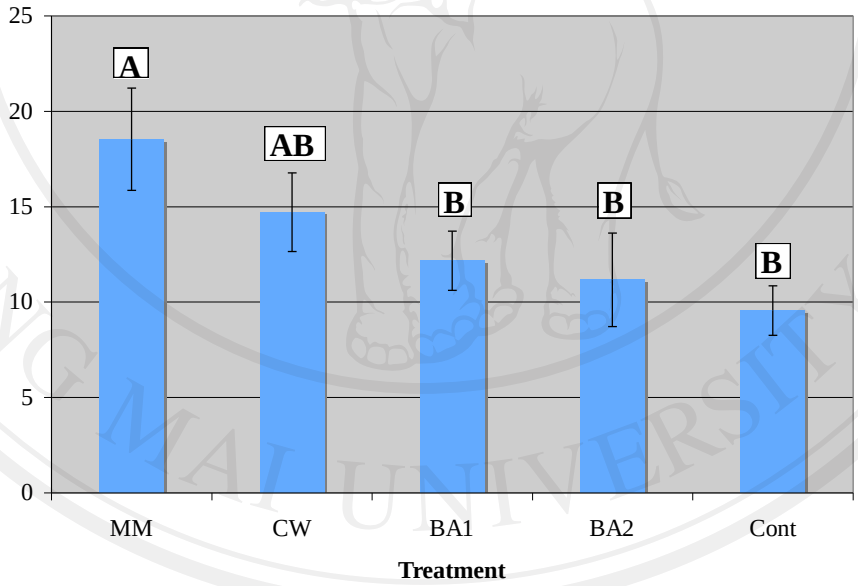
4.2.3 Mean final circumference of plantlets

No significant differences occurred between sites, but there were significant differences between treatments.

Plantlets in the uplands had a greater mean final circumference than in the lowlands, but not significantly (Fig. 34a). Across sites, though, there were significant differences by treatments. MM treatment had a significantly ($P<0.05$) greater circumference than other treatments with the exception of CW (Fig. 34b).



(a)



(b)

Figure 29: Mean circumference of plantlets as a function of site (a) and treatment (b). Mean separation, indicated with letters above bars, by Fisher's LSD, with alpha = 0.05, using PDMIX800. LSD = 4.919(a) and 5.649(b). Sites: lowlands (LL) and uplands (UL). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error.

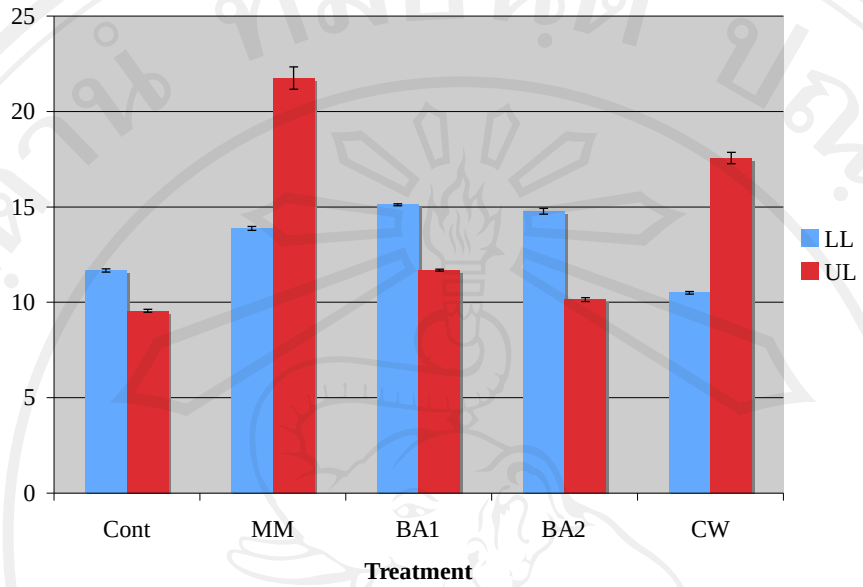
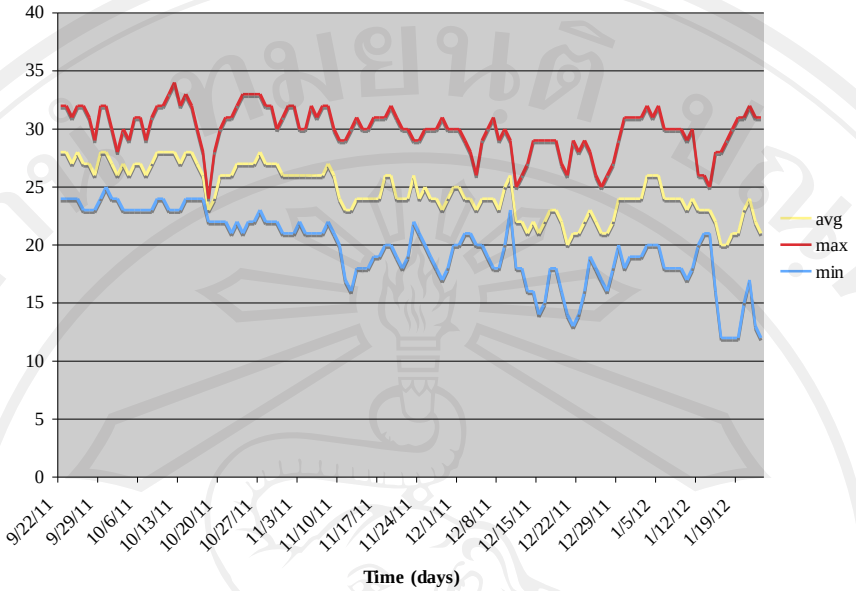


Figure 30: Mean final circumference between site and treatment. Sites: uplands (UL) and lowlands (LL). Treatments: control (Cont), plants produced from stem fragments (PIF), benzylaminopurine 10^{-2} M (BA1), coconut water (CW), split-corm (SC), and benzylaminopurine $5x10^{-3}$ M (BA2). Vertical bars indicate standard error; treatments were analyzed within sites.

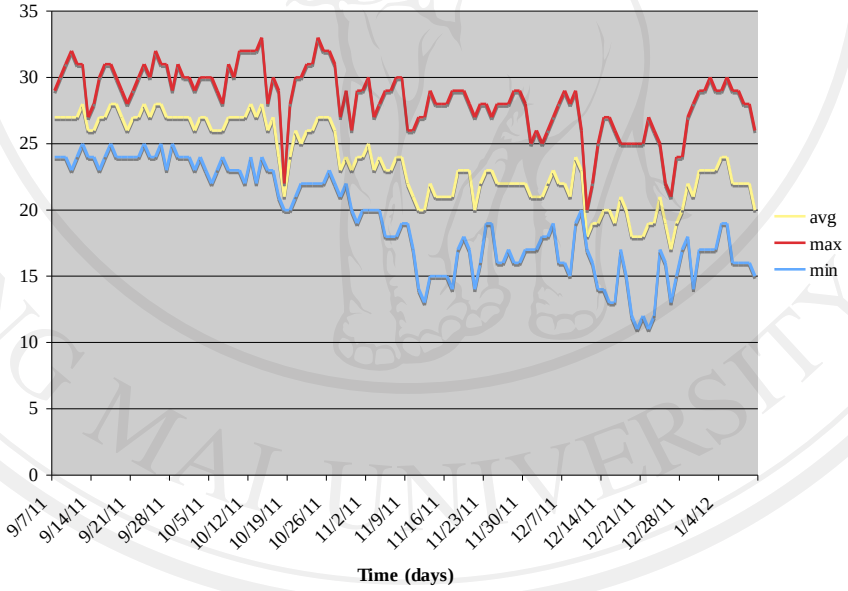
4.2.4 Other factors

4.2.4.1 Temperature

There were higher maximum temperatures in the lowlands and lower minimum temperatures in the lowlands, with average temperatures slightly higher in the lowlands than in the uplands. There is also a definite downward slope as the season changes from warm to cold (starting around mid-November) (Fig. 35).



(a)



(b)

Figure 31: Temperature measurements for lowlands (a) and uplands (b) field study (taken from Wunderground, 2012) with average, maximum, and minimum daily temperatures.

4.2.4.2 Pests, diseases and flood

There were some signs of pest and disease presence in the plants used in the field studies, although not many were observed. Of the two or three affected plants, the problems found were: weevil damage from the *Odoiporus longicollis* Oliver adult and larva and fungal damage (Fig. 36). Weevil damage was evident by through extensive tunneling in some pseudostems, which sometimes killed the mother plant. Fungal damage was evidenced by the rotting of a banana pseudostem from the base of the plant. One CW treatment from one block in the uplands was removed from the analysis as a result.

Early in the study (the end of September), high flooding in the lowlands caused some damage to some of the bananas, knocking down mother plants and exposing treated corms. Two of the treatments (BA2 and CW) from one block were removed from the analysis as a result.

(a)  (b)  (c) 

Figure 32: Pest problems in field study: *Odoiporus longicollis* Oliver (a), banana stem damage caused by larvae (b), and fungal damage to pseudostem (c).

4.2.5 Field discussion

In conclusion, site did not make a significant difference in the number of emerged plantlets, cumulative days to emergence, cumulative GDD to emergence, or final circumference or corms. Between treatments MM had significantly greater circumference than BA1, BA2 and Cont, but not CW.

It is possible that MM treatments increased plantlet circumference because the inherent mat age of mother plants used for this treatment were younger than mats for other treatments. Older mats produce more water suckers and water suckers have a smaller pseudostem circumference than sword suckers (Robinson, 2010) leading to weaker plants. Also, when damaged, corms tend to produce broader leafed suckers, may induce reduced plantlet circumference (Simmonds, 1966). It is possible that in the MM treatment, mother plants were young and suckers were not damaged, thereby producing suckers with larger circumferences. Although, Cont, BA1, BA2 and CW treatments utilized older mats, perhaps producing more water suckers. Additionally, for these treatments, the corms were damaged (with the exception of Cont), thereby encouraging plantlets with water sucker traits to emerge.

The lack of significant difference between treatments indicates that there were factors preventing treated plants from producing more plantlets than untreated plants. It is likely that the apical dominance of the older mother stalks had too much influence over the mats, preventing treated suckers from producing lateral bud formation. Because of apical dominance, in a stalk's lifetime of 12-14 months, it will only produce 5-20 suckers (Singh, 2011). Research suggests that in addition to the apical dominance exerted by mother plants over suckers, suckers in the same mat also exert dominance over axillary buds and slow their growth (Pillay, 2007). Both

Macias (2001) and Dr Rowe (Price, 1999) experimented, using young mother plants. The age of banana mother plants in our research was restricted to older plants because it was difficult to find fields with enough young mats and few, small suckers. Ideally, we would have been better to have been able to plant a field of bananas 10 months prior to treatment, starting with plantlets of known provenance and consistent size and age.

Additionally, initial sucker size may have had an impact on determining how effective the treatments were. Macias (2001) used suckers 15-20 cm in height, but Osei (2005) found that smaller suckers produced fewer plantlets. It was not possible to evaluate this from the field study, as there were not adequate numbers of large and small suckers to determine significance. More research is needed to better understand this correlation.

Pest, disease, and flood problems may have played a role in determining growth of plantlets. In tissue culture research, axillary shoot production influences the number of established plants, and has a tendency to be lower in disease infected cultures than in healthy ones (Preece, 2008). The extent of damage caused by pests and diseases was not completely known in this research, as they are primarily unseen until far advanced in their damage (Azam, 2010; Sadik, 2010). Those with extensive damage were removed from the study.

Overall, it appears that temperature played little role in determining the success of treatments. Otherwise, there should be greater differences between sites, especially with respect to cumulative GDD to emergence, since temperatures differ between uplands and lowlands. Ambient temperatures were likely not extreme enough to affect the growth of the plantlets because they stayed within the optimal range (Singh,

2011). It is possible that water was a limiting factor, because it is the next most important factor in plant growth after temperature (Robinson, 2010). The study started close to the end of the rainy season and no irrigation was used in the fields and there was little rain after October. Ideally, treatments should be applied at the beginning of the rainy season to prevent water from being a limiting factor. More research could also be conducted that compares irrigated fields to rainfed in order to determine the effects of watering on treatments.

Although there were not significant differences between treatments and between sites, it may still be beneficial to pursue low-cost field propagation methods. There are other promising examples of macropropagation found in literature that should be included in further studies (Simmonds, 1966; Faturoti, 2002; Baiyeri, 2005; Pillay, 2011) although under different settings, constraints and with different banana or plantain cultivars. Further research should determine if they have a place in Thailand banana propagation.

Finally, more time may have been needed for data collection in the field study to better determine the effect of the different treatments on efficacy of propagation. In the literature plantlets emerged and data was collected after 3 months (Macias, 2001) and 4 months (Price, 1999), but, in this field study, plantlets were still emerging after 90 days. Perhaps running the experiment a few more weeks would have resulted in greater differences between treatments.

Recommendations:

- It is important to start with clean, healthy planting material when establishing a field for commercial propagation.
- Treatments should be initiated at the beginning of the rainy season to eliminate

water as a limiting factor.

- It is possible that more time was needed to determine how effective different treatments were, as there were still new plantlets emerging at the end of the study period.

Further research is needed:

- to better understand the correlation between increased circumference and the MM treatments; more studies could be completed that compare age of mats to characteristics of emerging plantlets,
- to determine the correlation between corm size and effectiveness of treatments, and
- to determine whether water played a role in results; future studies should be begin at the start of the rainy season or should compare the effect of irrigated and rainfed systems on treatments.

Although these results were different than our hypothesis, they should encourage further research on macropropagation of bananas. For a worldwide crop as important as bananas, it is crucial that farmers are aware of the necessity for quality vegetatively propagated plants and understand the future negative impacts of using substandard material. Since local markets rely on pig production from low-income farmers, it is important that farmers have access to inexpensive, quality material to provide adequate, sustainable nutrition for their animals and make a profit. In order to provide these materials, this macropropagation research must continue.