ILRI Forage Diversity:

A study of seed production in the greenhouse, field, and farm



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Introduction

I grew up on a farm in southwestern Iowa, chasing massive Angus beasts down the road, bottle-feeding orphaned sheep, and tending to chickens and geese. When I was 12 my family moved to a suburb of Des Moines, and suddenly my backyard shrank rather drastically. My perspective on agriculture shifted, as I began to buy eggs in the grocery store and frequent farmers' markets for my strawberries. Since Johnston is a Mecca for industrial agriculture, the home for Pioneer Hi-bred and John Deere, I had assumed the inhabitants would be at least relatively attuned to agriculture, but I found that many of my new friends gave no thought to where their steak began its journey**.** From my table in the high school cafeteria, all I could see were rows of hybrid corn and soybeans, but the people around me would have easily believed GMO to mean 'Green Monstrous Orangutan.' I learned that there is a severe disconnect between Americans and their food: its origin, processing procedure, impact on our world, even for those barely removed from the farm.

As Americans, it's terribly easy to cruise the sparkling aisles of Hy-vee or Kroger's, sweeping up peanut butter, tomatoes, and hamburger without a care, because there's a façade of cleanliness in the American supermarket. There aren't any warning labels about palm oil production destroying gorilla habitats, or the gallons of oil and water consumed for the production and transportation of those California tomatoes, or the environmental implications of our corn-fed, antibiotic-abundant beef system. When I heard about the World Food Prize Foundation and its Global Youth Institute, I was thrilled to discuss these issues and so much more with like-minded students and renowned experts. I attended in 2012, and my view shifted again. The institute opened my eyes to the fight for food security world-wide; internationally, there are concerns, issues, and calamities in food production that I'd never imagined. I saw how limited my agricultural background truly was. It inspired me to apply for the Borlaug-Ruan International Internship so that I could experience the lives of those across the world and how food was a factor. In addition, the internship offered the opportunity to see what I can do to help.

I feel incredibly grateful and very lucky that the World Food Prize Foundation chose me as a 2014 Borlaug- Ruan intern. I was sent to the International Livestock Research Institute (ILRI) in Addis Ababa, Ethiopia. More specifically, I worked with Dr. Jean Hanson in the Forage Diversity department. Forage Diversity and Genebank have been in operation at ILRI for over 30 years- and for good reason. It is estimated that 70% of households in Ethiopia are at least partially supported by livestock, and that the country of Ethiopia has the highest amount of livestock in Africa (Kimball). Furthermore, the livestock sector takes up 45% of Ethiopia's agricultural GDP ("The Contribution of Livestock to the Ethiopian Economy"). These factors place a great importance on forage characteristics, as the structure of livestock in Ethiopia is dependent on grazing Grasses and legumes are the primary food source for Ethiopian livestock-cattle, sheep, and goats, and they will wander anywhere and everywhere for a meal (Mengistu). This is semi-nomadic system is a set-up frequently seen across the African continent. Thus, the work performed in forage diversity and development is integral to the future of food security in Ethiopia and Africa.

It's a far cry from the large-scale industrial meat production of the west, but this system of free-for-all, perpetual grazing has taken its own toll on the environment. After thousands of years of this method, as well as a constantly changing land-tenure system, it is not surprising to see that soil erosion, deforestation, and decreasing soil fertility and structure is rapidly emerging ("Land Degradation and Its Impact on Amhara Region"). Declining land productivity is being met in turn with an increasing population, placing yet a greater demand on remaining arable land ("The Change Drivers"). Greater amounts of acreage are being used to grow grains like teff, maize, barley, sorghum, and wheat to feed humans, replacing grazing land, while at the same time livestock numbers continue to grow (Mengistu). One piece of a more sustainable solution lies in developing the most efficient forage grasses and legumes for farmers, as well as education concerning the best use of available forage.

While Ethiopia is the native home for a diverse variety of forage, genetic erosion has become commonplace with the lack of interest in conservation in the past ("Forage and Pasture Field Genebank"). In recent history, however, there has been a renewed pursuit of maintain this diversity, which is where genebanks become crucial. The Addis Ababa-ILRI forage genebank is home to an immense amount of forage germplasm. These seeds are sent to various research stations, but also used for seed production at the ILRI test sites around Ethiopia. This can take the form of large-scale regeneration in fields, more precise production in smaller plots, or individual care of a few pots in greenhouses- often for seedlings and transfers.

My work, as I spent eight weeks in Forage Diversity was focused on seed production. My time was consumed in literature research, background study of plant biology, anatomy, and genetics, devising experiments based on my research, as well as assisting others in their work and viewing the different cogs involved in forage diversity. I learned about seed production in the field- for regeneration, in accessions- for research, and more. Furthermore, I was assigned a personal project wherein I studied two forage legumes that have been growing in the Addis Ababa ILRI greenhouses for many years, but haven't produced any seeds. My job was to find out why.

Legume Background

Trifolium ambiguum

Also known as the Kura clover or Caucasian clover, *trifolium ambiguum* has been heralded in many parts of the world as a miracle forage (Henry). Found originally in parts of Europe and Asia, it has been exported and grown widely since the early 20th century. It is extremely well adapted to a range of climates, from marshy valleys to towering mountaintops. It has not been domesticated in its native land, but there has been a recent upsurge in its popularity from Australia to America (Frame). *Trifolioum ambiguum* boasts a high grazing quality due to its method of growth. Stem production usually only occurs in the first year and from then in, leaves sprout from short stems. This gives it a high leaf: stem ratio, meaning the protein levels are between 18-25% and fiber from 25-40% in temperate conditions (Peterson).

The leaves of the Kura clover are also larger than its more famous cousin, the red clover, and have no hair. This aids the digestibility of *trifolium ambiguum*, making it more easily digestible than other legumes like lucerne, red clover, birdsfoot trefoil and crown vetch (Albrecht: "KURA CLOVER: A Promising Pasture Legume for Wisconsin"). A long-running trial of Kura clover at the University of Minnesota found the NDF, ADF, ADL, and IVDMD at 30, 27, 3, and 87%, respectively, all of which indicate that the legume is a quality performer. It may be the most digestible of all leguminous forage, as it has been shown to have a mean IVDMD averaged over 1981 harvests, 15 units higher than any other species (Peterson). All in all, it has high protein levels, moisture levels, nutritive value, and digestibility.

In addition, Kura clover is unique in that it develops a massive root biomass system composed of rhizomes. It has been purported to generate up to 9 tons of rhizomes and roots per acre (Pedersen). Given that *trifolium ambiguum* spreads underground lineally through the production of rhizomes and its tendency towards horizontal rather than vertical growth, it has the ability to spread rapidly and reproduce indefinitely once established (Albrecht). Furthermore, the legume has been shown to be incredibly hardy, performing especially well in cold climates, but also with a high resistance to heat and drought (Frame).

Fig. 1.1

This table displays the relevant traits of Kura Clover with its most prominent forage competitors:

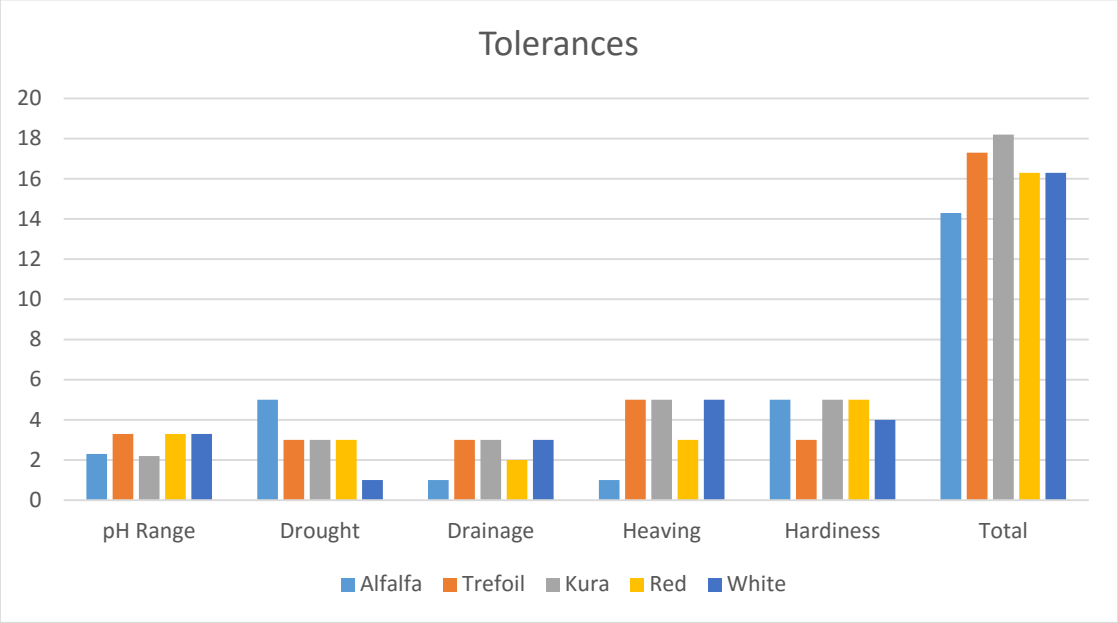
		Tolerances			
Species	pH Range	Drought	Poor Drainage	Heaving Potential	Winter Hardiness
Aflalfa	6.1-8.4	Excellent	Poor	High	Excellent
Trefoil	5.1-8.4	Good	Good	Low	Good
Kura Clover	5.1- 7.3	Good	Good	Low	Excellent
Red Clover	5.1-8.4	Good	Fair	Moderate	Excellent
White Clover	5.1-8.4	Poor	Good	Low	Very Good

Adapted from Forages, 6thEdition and NRCS Pasture Handbook (Moore)

Fig. 1.2

The following chart shows the above information with numerical values attributed to each category laid out in Fig. 1.1:

Poor(1), Fair(2), Good(3), Very Good(4), Excellent(5); High(1), Moderate(3), and Low(5).



Furthermore, the underground biomass produced by *trifolium abiguum* makes it a fantastic grazing legume because it has a minimal negative response to repeated cutting (Albrecht). In a trial in Rosemount, MN, even after 4 cuts in one year, the final stand of Kura Clover was 91%, nearly three times the next closest sample- alfalfa at 38% (Peterson). Its rhizomial nature sets *trifolium ambiguum* far apart from other popular perennial legumes like alfalfa and birdsfoot trefoil. Another factor in its persistence is that the system produces buds at various depths in the soil, allowing for a thorough recovery from damage, whether it be from a ruminant, machine, or frost (Albrecht).

Because of the resources required to develop such a large root system, however, it is quite difficult to establish Kura clover. It is commonly said, coined by Dr. Gordon Marten, that *trifolium ambiguum* "sleeps in the first year, creeps in the second year, and leaps in the third year (Peterson)." It is a legume that requires patience and time, but can be well worth the wait. A lamb grazing experiment with the University of Minnesota at St. Paul found that the days of grazing and tons of dry matter per acre was slightly higher with birdsfoot trefoil than Kura clover initially. By the fourth year, however, "Kura clover had double the grazing days and gains per acre of those of the birdsfoot trefoil monoculture.

Fig. 1.3

	1986		1987		1988		1989	
	DM	Lamb	DM	Lamb	DM	Lamb	DM	Lamb
	t/ac	Days/ac	t/ac	days/ac	t/ac	days/ac	t/ac	Days/ac
Birdsfoot trefoil	1.8	1532	1.8	1868	1.6	1350	.5	1009
Kura clover	1.4	1426	1.3	1750	1.5	1580	1.3	2099
BFT-KC mix	1.8	1571	1.7	1936	1.3	1724	1.3	2057

Source: Sheaffer et al., 1992. Agron. J. 84:176-180.

Trifolium ambiguum can be frustrating to establish because of its low seedling vigor. As Ulf Kintzel, of the Small Farms Program at Cornell University put it, "Planting Kura clover was a $300 lesson. I have no stand of Kura clover after seeding it 3½ years ago…given its price and the difficulty to establish it, compared to the price and how easily white and red clovers can be established, I doubt I will give Kura clover another try." As shown in above fig. 1.2 however, it can be beneficial to put forth an effort if it will result in Caucasian clover establishment. There are many environmental factors which affect the legume's germination and reproduction, which may have been a cause or two of Kintzel's trouble.

Firstly, the process of scarification has been shown to increase germination of trifolium ambiguum between 40 and 50% (Hannaway). Scarification is the process of scratch, soaking, or puncturing seeds to ensure water can permeate the shell that protects germplasm during dormancy. Due to the low-seedling vigor, it is imperative that it be sown only with other non-competitive grasses; an open sward canopy is absolutely necessary (Frame). Consequently, it is recommended that it be seeded with a legume like birdsfoot trefoil as it is a much quicker germinator and will help to control weeds (Hannaway).

The recommended seeding rate for Kura clover is 5.5-10.5 lb/acre, erring towards the lower side when sowing for seed production (Hannaway). Density can affect the moisture level, root system development, and reproductive capabilities. In a trial conducted by Shimin Fu, he found that there is a negative relationship between density and seed yield, with the lowest-density areas producing the most seeds per plant. Thus, the samples of *trifolium ambiguum* which produced the most seeds per acre were those planted at a medium density: 10.8 plants m^{-2}. There is a positive correlation between seed yield and root dry mass, as the number and size of secondary crowns will determine how many seeds are produced. "[This] in turn depends upon the size of the root system prior to reproductive development (Fu)." Therefore, each individual plant will produce more per plant with more space, but it is more effective to choose a medium density, as there are more plants to harvest from, resulting in a higher yield overall.

Kura clover does best in soils with a fine to medium texture, can handle 20-50 inches of precipitation annually, and is shade and saline intolerant ("Trifolium Ambiguum"). In addition,

trifolium ambiguum is self-incompatible, so it must be cross-pollinated, usually by honey bees (Hannaway). Kura clover also has evolved in a variety climates, so there are several different genetic forms, including diploid, tetraploid, and hexploid. The seed size, adaptability, and seedling vigor as well as rhizomes and flowers increase with ploidy, so the correct genetic form must be chosen for the environment (Frame).

There are several external stimuli that have an effect on flowering. For example, "if plants are kept in the wrong day-length, they remain vegetative indefinitely." Flowers were produced in greenhouse with a 17-hour photoperiod, suggesting *trifolium ambiguum* responds well to a long-day schedule (Fu). This is not surprising, as Kura clover is native to areas relatively far from the equator. Thus, they would be adapted to long summer days and winter nights.

This native environment also indicates that the legume has a vernalization requirement, meaning that it must be exposed to lower temperatures to trigger flowering. "Flowering in most cultivars [of trifolium ambiguum] does not occur until the second season after induction by low temperature in the winter (Taylor)." Finally, flower initiation has been shown to be earlier at lower elevations, suggesting that higher temperatures may aid flowering. Kura clover tends to produce better in colder climates, but this finding means that early exposure to warmth may speed along the reproductive process (Hannaway). The quality of light the plants experience is also important, as there has been shown to be more inflorescence production under a higher light intensity (Fu).

Since the underground root system plays such an integral role in the plant's health and reproduction, there are several relevant factors which affect its growth and development. While nitrogen is abundant in our atmosphere, its form, N_2, is unusable by most living organisms, though they need nitrogen to survive. The process of nitrogen fixation, which changes N2 to the usable form NH3 is performed in nature only by bacteria. Thus, bacteria can form a symbiotic relationships with plants wherein the bacteria is provided with nutrients and energy while the plant receives fixed nitrogen. This most frequently occurs between legumes and the bacterium rhizobium. It invades the plant's roots and multiplies, forming nodules: small outgrowths which will indicate nitrogen fixation is taking place when it turns pink/red. The color is caused by leghemoglobin, which regulates oxygen flow (Lindeman). If a legume isn't flourishing, it may be due to a nitrogen deficiency, and rhizobium fertilization may be necessary. Because of the low seedling vigor in *trifolium ambiguum*, many experts even think of the treatment as essential (Albrecht: "Establishing Kura Clover Stands").

Indigofera Whigtii

Indigofera whigtii is also a perennial forage legume. There is far less information available about this species, however, and it is found in far fewer places- India, Sri Lanka, China, Cambodia, and Nepal (“Indigofera Wightii:” ILDIS).

Fig. 1.4

(“Indigofera Wightii”: Discover life)

It generally grows at a lower altitude than Addis Ababa: between 600 and 1800 meters and is native to sandy, sloping areas and grasslands ("Indigofera Wightii:" *South China Botanical Garden Herbarium)*. This suggests that the legume would be advantageous as forage in Ethiopia, as surrounding areas, especially the Rift Valley is well within the elevation range. Furthermore, as it has adapted to grow in sandy and sloping areas, it should be able to flourish in conditions that aren’t necessarily ideal and in a land-efficient manner.

Reproductive Issues

Trifolium Ambiguum

There are three pots of *trifolium ambiguum* in the ILRI forage diversity greenhouses, and as of the 12 June 2014, they are languishing: short and pale green, with many shriveled, browning leaves. At this point, after 15 years of accumulating underground rhizomes, these plants should be in much better shape.

Fig 1.5- *trifolium ambiguum*

Fig 1.6- three pots

Using the research I had compiled, I was able to determine some possible causes for their current state. Firstly, these plants were scarified prior to planting and germination, and as they are planted in isolation, there is no competition concern. I do believe that density may be an issue, however. Since seed production is so linked with the size of the legume's underground rhizome mass, I believe the small planters may be limiting its ability to reproduce. Thus, the existing plants should be moved to larger pots which can support a root system which will sustain flower and seed production.

The most likely factors in the success of these plants lie in the light and temperature the plants are receiving. The climate of Addis Ababa, ETH is sub-tropical without any major change in temperature throughout the seasons. Thus, it is likely that ILRI's sample of *trifolium ambiguum* have never been exposed to a vernalization of cooler weather. Without this induction, is not possible for flowering to be initiated. I recommend a vernalization treatment that would mimic the climate of the plant's source of origin: Asia Minor, Crimea, and Caucasian Russia.

Though vernalization has been studied for nearly a century, “only a few comprehensive vernalization studies have been conducted on cold-requiring perennial species (Fausey).” The researchers at Michigan State University found that most perennials could be adequately vernalized at temperatures between 0 and 16°C, from anywhere between 4 and 15 weeks, with longer trials generally required for plants vernalized at higher temperatures. In addition, the longer periods tended to produce a greater response, with more flowers produced in less time post-vernalization. In the MSU study of spring perennial vernalization, they also disclaimed, that “all species could be effectively vernalized at temperatures of 41°F to 46°F for four to six weeks, although this was not necessarily the optimum temperature or required duration for maximum flowering (Fausey).” Based on this research and the climate of Kura clover’s homeland, I suggest a vernalization treatment of one *trifolium ambiguum* plant.

Furthermore, the proximity of Addis to the equator gives about a 12-hour day, much shorter than the recommended 17 hours. Therefore, in the ‘minds’ of these plants it would appear to winter all year long, preventing any flower initiation, as it’s closely linked with day length to ensure a profitable regeneration. Otherwise, an early bout of warm weather could trick plants into flowering, only to be killed by a late freeze. Since Kura clover should then flower in response to the lengthening days of early summer, an elongated photoperiod would be beneficial.

The phytochrome is a light-sensitive protein in plants that regulates many essential processes including circadian rhythms, germination, and time of flowering. It responds to the red and far-red ends of the spectrum, as opposed to cryptochromes, which react to blue light, and other photoreceptors (Sharrock). In the early 1950s researchers Lewis H. Flint and Flint and E. D. McAlister of the Smithsonian Institution discovered that exposure to red light (wavelength ~ 620-740 nm) promoted germination while far-red light (710-850) stalled it. Even more interestingly, if the seeds were hit with far-red light, all that was needed was another exposure to red light for germination, even in with 100s of these cycles. Conversely, their subjects flowered only when exposed to far-red light last (Adams).

Indigofera Wightii

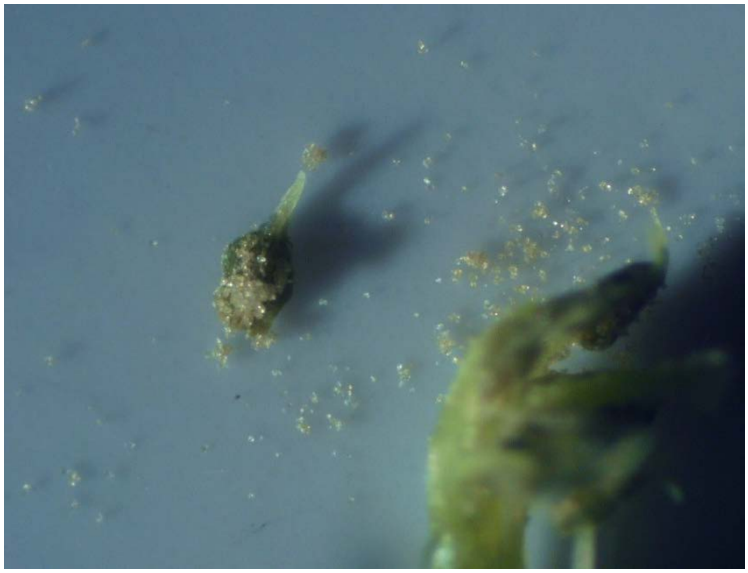
While there is little literature available concerning the *indigofera wightii*, there are an abundance of flowers produced by the one housed in ILRI greenhouses, so I was able to study them and draw conclusions based off of dissection and various tests.

Fig 1.7

Tests/Results

First, I dissected ten flowers at all stages of maturity, to see if there was a biological issue at fault for the lack of seeds. I found that each flower contained healthy-looking ovules, as well as shiny, white stigma. The ovules were always abundant and to scale with the style they were encased in. Additionally, they were always uniform and their appearance never changed regardless of how mature the flower was. Overwhelming, there was a plethora of pollen in the anthers of each flower, even flying out in a miniscule golden cloud when the pistil is popped out of the perianth.

Fig 1.8



In several flowers, however, I discovered anthers were occasionally on filaments which were shorter than the style.

Fig 1.9

Fig 2.1

This could prove to be a problem if it means the pollen is unable to reach the stigma for germination. This seems more likely when considering the growth pattern of the inflorescences. As is shown in Fig 2.3, the pistil grows out and up, leaving the stigma as the highest point. Thus, if the stamen are shorter than the style, gravity will pull stray pollen down and away from the stigma.

If this were indeed the issue with reproduction, it should be fixed with hand-pollination. I conducted this trial on 13 June 2014 by collecting pollen on a short paint brush and transferring it to the stigma of a different flower.

There were a couple concerns involved with this method, however. Firstly, in order to reach the stigma, it was necessary to pop the pistil out of its protective petal shell, leaving the pistil vulnerable to wind, rain, and heat damage, any of which could interrupt the germination process.

Fig 2.2

Fig 2.3

Fig 2.4

Secondly, the microscopic size of the flowers made them difficult to manipulate without damaging them myself.

Accordingly, I conducted several other trials wherein I turned the inflorescence upside-down, so that the pollen would be pulled down by gravity. I then proceeded to tap and firmly stroke the petal sheath around the androecium and gynoecium. This way the reproductive parts could stay protected while I ensured the stigma came into contact with pollen grains. This was done on the 17th, 23rd, 27th of June, and 7th of July. In the weeks that have ensued, however, I have found no evidence of seed pods forming. Instead, the flowers have continued to grow brown, wither, and fall.

While performing dissections, I have found pollen present on about half the stigma. While I can't know if this is because pollen has been transferred to the stigma when the flower was plucked, carried from the greenhouse, and/or dissected, it would also indicate that there is an issue with the germination process besides contact between stigma and pollen. This may mean that the plant's pollen is not viable.

To test this, I performed several tests: a germination of the pollen in sucrose solution, a stain test, and a stain test of germinated pollen. The first was conducted by brushing pollen grains and anthers into several drops of the sucrose solution, then heating it on an incubator for several hours (see Appendix E & F). I observed it three times: four, seven, and twenty-four hours after commencing. However, after many hours I found only four pollen grains which had definitely germinated, with long clear tails that protruded from the edge of the grain. As there were hundreds of grains present, this result suggest the pollen has a low rate of germination.

To check this result, we mixed a stain solution using brilliant green, orange g, and acid fuchion (see Appendix G & H). I collected pollen and dropped it into several drops of the stain solution on a microscope slide. Then, using an alcohol-based spirit lamp, I held the sample of a flame until it boiled, about 30 seconds. After letting it sit for 10-15 minutes, I observed the results. Pollen grains that are viable should show up as darker than the others: stained. In order to get a random sample that I could generalize instead of recording the results of each grain, I noted the outcomes of each window of slide I could see through the microscope in a proportion of stained/total. I did this ten times for each sample. The resulting proportion was 174/273, or 67.3%.

Shortly thereafter, I combined both experiments by staining germinated pollen. This required a germination of pollen in sucrose solution on an incubator, followed by an addition of stain, which was then boiled. In this test, I found many stained pollen grains- it appeared to be a similar proportion to the last time. In addition, one pollen tube was found, but it was not stained.

Since these results were inconclusive, and nearly contradictory, I set about a fourth test, also a kind of stain. This test, using Analine Blue Fluorochrome, requires several steps to germinate, preserve, soften, and finally stain the pistil (see Appendix I & J). After this is done, the pistil can be squished and viewed under a microscope with UV light, under which pollen grains and any tubes that have formed should fluoresce. I first practiced this procedure with samples from a nearby Sesbania tree, and then began the process with *indigofera wightii* pistils.

Three days later, they were ready for viewing. I had prepared 5 pistils, and they all looked generally similar. I observed that the tip of the style- the stigma, was highly fluorescent, and looked like it had dozens of shorts hairs protruding from it. It glowed bright green under the UV light, so this could be an indication that the pollen is germinating well, as the stigma was covered in pollen prior to germination. It was then left to sit for about four hours, so it in unrealistic to expect pollen tubes to be forming along the entire length of the pistil. What I observed appeared to be a plethora of pollen tubes extending towards the ovules, but I was hesitant because they also appeared similar to veins or vertical cells that would be present in a pistil. Furthermore, I

was apprehensive about the apparent pollen grains, as there didn't appear to be any nuclei present.

Due to the various concerns in this test, I conducted it again, beginning on July 18, 2014. This day I pollinated one pistil with its own pollen, and another I cross- pollinated with pollen from a different flower. They were left to germinate for 8 hours before I placed them in fixative. They were subsequently softened and stained with Analine Blue Fluorochrome. On July 24, 2014, they again fluoresced a bright green under UV light. This time, Dr. Jean Hanson was able to give an expert opinion on the appearance of the *indigofera wightii* pistils. She pointed out two obvious pollen tubes on the self-pollinated stigma, as well as one on the pistil of the cross-pollinated flower. This, along with the appearance of fluoresced pollen and the previous test results, suggest that the pollen is definitely viable.

Early on, I was reluctant to say that the plant needs to out-breed. While I was unable to find any literature which definitively stated the reproductive tendencies of *indigofera wightii*¸ I researched other species in the same genus: *Indigofera,* and found that they all self-pollinate. This is underlined by the fact that all flowers I have examined have been hermaphroditic, with both stamens and pistils containing pollen and ovules, respectively, and no apparent reason for a lapse in functionality. It may be, however, that the *indigofera wightii* requires cross-pollination to reproduce. Unfortunately, there is only one plant here, so if this is the case, we will need to plant another to move forward with that line of research.

Finally, on July 24, 2014, Dr. Hanson and I conducted a final hand-pollination test. We collected pollen on a small paintbrush and transferred it to other stigma repeatedly. We observed that the pistil and androecium sometimes require quite a bit of coaxing to pop out of the petals. This may point to a reason it has not been pollinated by insects thus far. The shape and color of the flower suggest that it is in the business of attracting pollinators, but this function would seem to be counter-productive in that respect. Furthermore, once it does pop out, a small cloud of pollen explodes, going everywhere, except for apparently the stigma. It is also impossible to squeeze back into the protective shell once removed, which raises concerns as to its possible longevity- perhaps the pistil will dry out, be damaged by wind, or the pollen could be washed away when the plant is watered.

Regardless, since we know that the pollen is viable, we are hopeful this massive sweep of pollination will be enough to produce a seed pod. In the coming week the *indigofera wightii* plant should be closely observed so the response can be noted.

In addition, multiple spider mites and many aphids- along with their companion, ants, have been observed living off the *indigofera wightii*. This is also a factor that can affect plant growth and health. In order to better understand the pest, I took samples, studied, and researched the pests (see Appendix K)

Conclusion

While I was unable to decisively state the reason *trifolium ambgiuum* and *indigofera wightii* are not regenerating, I was able to eliminate a few possibilities and offer suggestions for the future.

Through the tests and experiments that I have conducted on the *indigofera wightii*, I was able to rule out a potential cause for its reproductive issue: a failure to set seed after flower production. Based on the results of a germination in sucrose test, brilliant blue staining procedure, a combination of the two, and finally an Analine Blue Fluorochrome stain, I determined the *indigofera wightii* pollen is viable, though at the most, about 2/3 of the time. To establish what is the cause for its regenerative failure will require further research. A possibility could be an incompatibility between the pollen and the ovules or style. This would prevent the male gametes from reaching the female reproductive organs, even if pollen is germinated and forming pollen tubes. This hypothesis is supported by the results of the ABF staining test, as the pollen tubes were rather short, even after an extended germination period, so no pollen was able to reach the clearly visible and apparently healthy ovaries.

As for the *trifolium ambiguum,* its poor performance and rate of growth are most likely due to environmental conditions, as they are less than ideal in Addis Ababa, ETH for the *trifolium ambiguum*. From literature research and observation, it can be concluded that the pots of Kura clover are in need of a longer day, a different quality of light, and a period of vernalization. However, these plants have been exposed to a longer photoperiod for over a year, but there have been no results. Therefore, I suggest a treatment of vernalization and far-red light.

Fig 2.5

One pot of *trifolium ambiguum* has been placed in an incubator on July 22, 2014. It is watered each day and kept at a minimum of 16 degrees Celsius and a maximum of 24. My suggestion is that there is an effort put forth to decrease the temperature further, as it has been shown that vernalization generally has a greater effect between 0 and 7.5 degrees Celsius

It is important that the actual cause for these reproductive issues is discovered, as *indigofera wightii* and *trifolium ambiguum* hold great promise for making a difference in the lives of Ethiopian smallholder farmers. In the visits to farms conducted July 14-17, none of the farmers surveyed were growing *Trifolium* or *Indigofera,* let alone my specific species, nor did they have any plans to cultivate them in the future. I believe, however, that they could both be incredibly useful as forage standards. The *indigofera wightii* has an ability to grow in sloping areas, which is important due to the hilly nature of the Ethiopian countryside, and sandy soils. *Trifolium ambiguum* has been shown as a leader among its peers in nearly every forage standard, meaning that it has the potential to increase the output and value of livestock raised for both fattening and dairy. Furthermore, it is incredibly hardy and long-lasting after it has been established, due to the extensive system of rhizomes developed underground. This means it has potential for success

and improvement in Ethiopia, as eastern Africa has suffered droughts in the past, and will likely be faced with such extreme conditions again as global climate change continues to shape our environment. Therefore, if solutions can be found to the legumes' lack of reproduction, they can be placed in the right environment and used to produce a greater quality of life.

Kayla and I also collected information from nine small-scale forage seed producers in a survey format while traveling around southern Ethiopia. The results are compiled in my full-length report and are on file in the forage diversity department and agribusiness administration for the FeedSeed Project. I hope that they will be useful in indentifying, monitoring, assisting, and training of seed producers.

This experience has been so much more than research and lab work, though I will take with me innumerable new skills in the scientific field. This internship has actually reminded me quite a bit of my time as a page in the Iowa Senate this past spring. It is easy for a person to stand at a distance and say that he or she understands the workings of congress and the rest of our government. It is not until someone is able to be there within the wheels and cogs that simplifications fall away and the true complexities become apparent. It has been much the same experience for me over the past eight weeks as I've been involved in the activities of the ILRI Forage Diversity department and see how day-to-day work progresses into weeks and months. I have been able to experience a range of responsibilities, including literature research, seedling germination, field planting, chemical lab work, experiment design and carry-through, including drawing conclusions and making hypotheses based on tests and research. Certainly, at the beginning of this internship, I could have told you what a scientist does, but now I have a much deeper grasp, rather than a shallow understanding.

Furthermore, this internship has opened my eyes to the possibility that this could be a career for me in the future. I've never thought of myself as someone who is good at science, though I now realize that's a horrendously broad statement to make, and is probably influenced by a good deal of social factors. Now I can see that with hard work I would be happy in this field. This experience has further solidified my passion and drive to fight for global food security, but while I previously thought I would be able to contribute only through social sciences, I now see otherwise. I have been inspired by the people I work with and farmers I've visited to make this, advancements in global resource management, my life-long work.

Since a very young age, I've always dreamed of traveling. I've known since I was a little girl that I need to see how other people live, what they eat and wear and say, but in the back of my mind I was worried I wouldn't be able to survive. I'm rather close with my parents and siblings, so the possibility of homesickness was a factor I couldn't ignore. I've always been frightened that an attachment to home, and inability to adjust to new circumstances would keep me from experiences I seek. I am forever grateful for the Borlaug-Ruan International Internship because it showed me that I will be just fine. I feel so much more confident than I was eight weeks ago, not only about my capability in chemistry, or my passions in life, or even my tolerance for change, but also as a person. I feel extremely fortunate that I was given the chance to spend two months by myself, in a new country with new people, examining and reconfiguring my identity. And I can't wait to travel again.

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Regeneration Guidelines

Forage grasses

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Introduction

Grasses are members of the Poaceae family. There are about 650 genera and 10,000 species widely distributed throughout the world, with over 600 species commonly used for grazing and livestock feeds. Grasses are herbaceous annuals and perennials, often with rhizomes or stolons, with species ranging in height from a few centimeters to over two metres in height. Flowers are composed of spikelets in a panicle or in racemes or spikes. Two sterile glumes support one to many florets. Each floret is composed of two bracts, the lemma and palea which enclose a small flower. While many grasses are out-crossing and wind-pollinated, a combination of apomixis and sexual reproduction is also common in the Paniceae tribe. Most forage grasses produce seeds except for few shy seeders that rarely or never produce caryopses and need to be maintained in the vegetative form.

With such a wide range of diversity, specific conditions and methods are required for each species. These general guidelines are only indicative and specific information for each species should be sourced from literature.

Preparation for regeneration

When to regenerate

- When seed stocks are less than 1000 seeds.
- When percent germination is reduced to 65% or 85% of initial germination for wild species with low initial viability (FAO/IPGRI, 1994).

Other precautions

A population size of at least 100 plants should ideally be used for regeneration of out-breeding grasses in order to maintain genetic variation. For those reproduced vegetatively, one plant is sufficient to maintain the genetic variation but more plants are needed to mitigate the risk of loss of material. There are no reported transgenes in forage grasses.

Choice of environment and planting season

Field selection and preparation

- Select the environment and soil type best suited for the species.
- Plough and disc-harrow to obtain a well prepared and level seed bed prior to planting. Remove any weeds or other grass.

Planting season

- Plant in the rainy season to avoid water stress and ensure good establishment.

Method of Regeneration

Planting layout, density and distance

- Aim for a final plant number of 100 in plots of about 25 m^2 for out-breeders propagated by seeds and 25 plants in plots of about 10 m^2 for vegetatively propagated grasses.
- Plant in rows, each row 50 cm apart with within row spacing of 50 cm giving a density of 25-100 plants per plot.
- Forages vary in their breeding systems and species are treated differently.
- For out-breeders use an isolation distance of at least 100 m between accessions. Plant accessions of other species that do not hybridize with the grass or of other genera between the plots of one species to increase the isolation effect.
- Use tall plants or artificial barriers as wind breaks to control pollen flow and prevent wind pollination.

Sowing method

1. Germinate seeds in Petri dishes in an incubator using the right conditions for the species.
2. As soon as the shoots start to emerge, plant the young seedlings individually in seedling trays or small pots filled with sterilized compost or forest soil.
3. Label the tray or pot with the accession number and planting date.
4. Keep the pots in a warm place away from direct sun but with good light intensity or in a greenhouse and cover at night to retain moisture.

5. Water carefully using a spray bottle so the soil remains moist but not wet.
6. Once seedlings are strong and growing well, place the pots outside so the seedlings can harden off; keep the soil moist.
7. Peg out the plots at the chosen row spacing and make holes at 50 cm along the row.
8. Transplant seedlings to the field, one seedling per hole, taking care not to damage the roots. Water after transplanting.

Fertilization

- Fertilizer application will depend on soil type and fertility. Follow local recommendations.
- It is possible to grow the crop without fertilizer, but apply phosphorous in the holes before planting using a fertilizer such as diammonium phosphate or other phosphorus rich fertilizer at 100 kg P/ ha. Apply 50-60 kg N/ ha as a top dressing at early flowering stage to ensure good seed quality and after every cut for perennial species.

Crop management

Weed management

- Early growth can be slow so weed by hand 4 weeks after establishment and then monthly.
- Eliminate off-types and plants growing off-row.

Irrigation

- Irrigate the field after sowing and then when needed. Do not allow leaves to wilt at any stage and ensure soil is moist at time of flowering.

Common pest and diseases:

Forage grasses are susceptible to many fungal diseases and few virus and phytoplasma depending on the species.

Pest and disease control

Spray with fungicide to control mildew during the rainy season or when using irrigation and with insecticide at the first sign of insect damage. Pay particular attention to army worm and spray at the first infestation.

Harvesting

- Inspect the field daily once seeds start to mature to determine the right date for harvest. Seeds often ripen unevenly and may shatter once mature so some seed loss in grasses is normal. Harvest when at least 50% of the seeds on the head are mature and before shattering.
- When equal number of seeds are required from each plant within an accession, hand harvest seed heads from each plant into individual cotton/cloth bags. Many grasses are stoloniferous and it is difficult to separate individual plants within the plot after some months of growth. In these cases hand harvest heads from the entire plot.
- Harvest by cutting the stems or heads when seeds are mature but before fully ripe seeds start to dehisce and shatter.

Monitoring accession identity

Comparisons with previous passport or morphological data

- Distinctive traits are specific for each grass species. Grass accessions are usually distinguished on the basis of tiller, flower, spikelet, pubescence and stem traits.
- Digital images are useful for identification and comparison.

Documentation during regeneration

The following information should be collected during regeneration:

- Regeneration site name and map/GPS reference
- Name of data collector
- Field/plot/nursery/greenhouse reference
- Accession number; population identification
- Source of seed
- Generation or previous multiplication or regeneration (if generation is not known)
- Preparation of planting materials (pre-treatments)
- Sowing date
- Field layout used
- Field management details (watering, fertilizer, weeding, pest and disease control, stresses recorded, others)
- Environmental conditions (altitude, precipitation, soil type, others)
- Emergence in the field or green house (number of plants germinated)
- Number of plants established and harvested
- Isolation method used
- Harvest date and method
- Quantity of seeds harvested/accession
- Comparisons with reference materials (record any identification numbers or references of any samples or herbarium specimens taken from this regeneration plot)
- Post harvest procedures

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1 Brachiaria ruziziensis florets with anthers ready for pollination in isolated plots.
Jean Hanson/ILRI

2 Chloris gayana producing seed ready for harvest.
Jean Hanson/ILRI

3 Seeds shatter as they start to mature in Brachiaria dictyoneura.
Jean Hanson/ILRI

4 Stoloniferous perennial grass plots should be harvested from all heads.
Jean Hanson/ILRI

5 Weeding of grass regeneration plots.
Jean Hanson/ILRI

Regeneration Guidelines

Forage legumes

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Introduction

Forage legumes are members of the Fabaceae family. There are about 650 genera and 18,000 species widely distributed throughout the world, with over 1500 species of legumes commonly grazed or utilized by livestock. Forage legumes may be herbaceous plants, shrubs or trees and annuals and perennials, with species ranging in height from a few centimeters to over 5 m in height. Leaves are often pinnate or trifoliate and flowers show a wide variety of colours, usually with 5 petals. Flowers of the subfamily Papilionoideae comprise a standard, 2 wings and 2 petals fused into the keel and flowers of the subfamily Mimosoideae are usually arranged in racemes or heads and petals are basally united into a tube. Pods usually dehisce into 2 valves.

With such a wide range of diversity, specific conditions and methods are required for each species. These general guidelines are only indicative and specific information for each species should be sourced from literature.

Preparation for regeneration

When to regenerate

- When seed stocks are less than 1000 seeds
- When percent germination is reduced to 65% (FAO/IPGRI, 1994)

Other precautions

A population size of at least 100 plants should ideally be used for regeneration of forage legumes in order to maintain genetic variation. Transgenes have been reported for alfalfa (Medicago sativa).

Choice of environment and planting

Field selection and preparation

- Select the environment and soil type best suited for the species.
- Select the field based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different pests.
- Plough to invert soil, followed by 2-3 passes with the disc-harrow to obtain a well prepared and level seed bed prior to planting. Remove any weeds or other grass.
- Most forage and pasture legumes species are small seeded in size. Planting depth should not exceed more than 3 cm.

Planting season

- Plant in the rainy season to avoid water stress and ensure good establishment.

Method of Regeneration

Pre-treatments

Many forage legumes have hard seed and require scarification before planting to allow imbibition. Use one of the following methods:

- Gently rub seeds between two pieces of fine sand paper until the seed coat is scratched
- Use tweezers with well defined square edges and apply gentle but firm pressure to the seed coat with the arms of the tweezers open to about 2 mm so that a small section of the seed coat will chip off
- Use a scalpel to chip the seed coat
- Pour boiling water over the seeds and leave to cool and soak overnight

Planting layout, density and distance

- Aim for a final plant number of 100 in plots of approximately 25 m^2.
- Plant herbaceous materials in 11 rows of 5 m long, with rows 50 cm apart and 30-50 cm between plants giving a density of 100-150 plants per plot.
- Plant smaller herbaceous materials closer together with rows 30 cm apart and 25-20 cm between plants in smaller plots to avoid large empty areas of soil between plants where weeds will grow.

- Plant fodder trees in larger plots, with rows 1-2 m apart and 1-2 m between plants or in single rows 5 m apart and 1-2 m between plants. Increase the between plant spacing for large trees.
- Forages vary in their breeding systems and species are treated differently.
- Use an isolation distance of at least 100 m between accessions. Plant accessions of other species that do not hybridize with the legume or of other genera between the plots of one species to increase the isolation effect.
- Bee proof pollination cages are the best solution to isolate accessions and prevent insect pollination to avoid outcrossing but are expensive.

Planting method

Direct sowing for accessions with large size or many seeds

1. Count or weigh the number of seeds to be planted per row and place in separate envelopes/bags. Allow two seeds per hole if enough seeds available because not all of them will germinate. If there are only a few seeds, plant one seed per hole.
2. Label the plot with the accession number, planting date and plot number.
3. Lay out the plots at the chosen row spacing.
4. Mark rows or holes for sowing about 2-5 cm deep, 30-50 cm along the row.
5. Check that the accession number is correct and place the corresponding envelope/ bag on the end of the row.
6. Open the envelope and place 2 seeds per hole at 30-50 cm along the row by hand sowing. Small seeds can be scattered along the row rather than planted in holes.
7. Cover with soil and lightly compact the row.

Seedling transfer for accessions with small numbers of seeds

8. Germinate seeds in Petri dishes in an incubator using the right conditions for the species.
9. As soon as the radicles start to emerge, plant the young seedlings individually in seedling trays or pots filled with sterilized compost or forest soil.
10. Label the tray or pot with the accession number and planting date.
11. Keep the pots in a warm place away from direct sun but with good light intensity or in a greenhouse.
12. Water carefully so the soil remains moist but not wet.
13. Once seedlings are strong and growing well, place the pots outside so the seedlings can harden off; keep the soil moist.
14. Label the field plot with the accession number, planting date and plot number.
15. Peg out the plots at the chosen row spacing and make holes at 50 cm along the row.
16. Transplant the seedlings to the field, one seedling per hole, taking care not to damage the roots. Water after transplanting.
17. Maintain accessions with few plants or weak seedlings in 8" pots for regeneration in the greenhouse.

Thinning

- If direct sown, thin to one plant per hole at 4-6 weeks after establishment when plants are growing well to give a plant density of about 100-150 plants per plot and avoid competition that will result in weak plants and low seed yields. When thinning, do not remove only smaller or weaker plants as this will reduce genetic variation. Thinning can be done at the same time as the first weeding.

Fertilization

- Fertilizer application will depend on soil type and fertility. Follow local recommendations.
- Apply appropriate Rhizobia innoculant when needed in the holes at planting.
- It is possible to grow the crop without fertilizer, but apply phosphorous in the holes before planting using a fertilizer such as diammonium phosphate or other phosphorus rich fertilizer at 100 kg P/ ha. Apply 50-60 kg N/ ha as a top dressing at early flowering stage to ensure good seed quality.

Crop management

Weed management

- Early growth can be slow so weed by hand 4 weeks after establishment or transplanting to the field. Cultivate between rows twice during early stages of plant growth. Ensure field technicians know what young plants look like so they do not mistake them for weeds.
- Eliminate off-types and plants growing off-row.

Irrigation

- Irrigate the field after sowing or transplanting and then when needed. Do not allow leaves to wilt at any stage and ensure soil is moist at time of flowering.

Common pest and diseases:

Forage legumes are susceptible to a wide range of virus and fungal diseases similar to many pulse crops.

Pest and disease control

Coordinate periodic field inspections with pathologists and virologists during the growing season.

Spray with appropriate chemicals to control diseases. Spray with fungicide to control mildew during the rainy season or when using irrigation and with insecticide at the first sign of insect damage. Rogue material infected with virus before flowering to eliminate the disease and incinerate.

Harvesting

- Inspect the field daily once seeds start to mature to determine the right date for harvest. Seeds often ripen unevenly and may shatter once mature so harvesting at the right stage is critical.
- When equal number of seeds are required from each plant within an accession, hand harvest seed pods from each plant into individual cotton/cloth bags. Many forage legumes are indeterminate in growth habit and it is difficult to separate individual plants within the plot after some months of growth. In these cases hand harvest pods from the entire plot.
- Collect the seeds from each plant in labelled cloth or paper bags with an additional label inside each bag. Use paper bags in dry climates only.

- Thresh the pods on a tarpaulin by gently beating or thresh small quantities from individual plants by hand using rubber threshing board; return the seeds to their labelled bag.
- Thresh larger quantities from full plots in a threshing machine.
- Ensure that seed mixing does not occur during threshing by thoroughly cleaning all equipment and implements between each sample. Wash and dry cloth bags between each use and avoid reuse of paper bags. Where resources are short and bags have to be reused, check carefully in folds and remove trapped seeds and use bags for different species so any mixed seeds can easily and quickly be identified and removed.

Post-harvest management

1. Clean the seeds of debris by hand picking, hand winnowing or using a seed blower.
2. Hand pick over the seeds in trays to remove any shriveled, discoloured, infected or damaged seeds from each plant. Incinerate the waste to avoid spread of seed borne diseases.
3. Compare the harvested seed with original seed of that accession for seed characters to check for mistakes/correspondence.
4. Take equal quantities of seeds from each plant and mix in one paper bag labelled inside and outside. Once you have all the seeds needed, discard any extra.
5. Retain the bags of each accession in temporary storage until seed drying.
6. Take a sample of the seeds and carry out seed health tests for common diseases. If the material is annual and the seeds are infected with seed-borne diseases and more original seeds are available for a second regeneration, destroy the seeds by incineration. If no original seeds are available, resow from the harvested seeds and use the correct fungicide to control the disease and obtain clean seeds. If the material is perennial and the fresh seeds are infected with seed borne diseases, treat the plants with the correct fungicide for the disease until no symptoms are seen on the plants and harvest fresh seeds. Destroy the earlier harvested seeds by incineration once clean seeds have been harvested. Thermotherapy and tissue culture can be used to remove virus but are time consuming and expensive.
7. If the seeds are free from pests and diseases, dry the seeds in low relative humidity at 15°C until they reach between 3-7% moisture content.
8. Remove the seeds from the drying room, weigh and pack directly into storage containers. Options for medium term storage include using plastic containers or cans with sealed lids for storage in environments with humidity control or laminated aluminum foil packets for storage in environments without relative humidity control. Use of laminated aluminium foil packets is more suitable for long-term storage. Seal the containers or packets immediately.
9. Sample and test the viability of the seeds and record the results following standard germination methods (ISTA, 2008). If viability is high, proceed to storage, If viability is low, reschedule the accession for a further regeneration from the original seeds.
10. Store seeds in the genebank at 5-10°C in medium-term storage or at -18°C in long-term storage.

Monitoring accession identity

Comparisons with previous passport or morphological data
Most forage legumes are distinguished on the basis of flower colour, flower parts, number of sepals, number of leaf pinnae in compound leaves, pubescence and stem traits.

Documentation during regeneration

The following information should be collected during regeneration:

- Regeneration site name and map/GPS reference
- Name of data collector
- Field/plot/nursery/greenhouse reference
- Accession number; population identification
- Source of seed
- Generation or previous multiplication or regeneration (if generation is not known)
- Preparation of planting materials (pre-treatments)
- Sowing date
- Field layout and density used
- Field management details (watering, fertilizer, weeding, pest and disease control, stresses recorded, others)
- Environmental conditions (altitude, precipitation, soil type, others)
- Emergence in the field or green house (number of plants germinated)
- Number of plants established and harvested
- Isolation method used
- Harvest date and method
- Days from sowing to flowering
- Number of plants established and harvested
- Quantity of seeds harvested/accession
- Comparisons with reference materials (record any identification numbers or references of any samples or herbarium specimens taken from this regeneration plot)
- Agronomic evaluation; agro-morphological traits recorded
- Taxonomic identification
- Post harvest procedures

References and further reading

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Loch DS, Ferguson J. (eds). 1999. Forage Seed Production volume II:Tropical and Subtropical Species. CABI International, Cambridge, UK.

1 Flowers of Vigna vexillata (subfamily Papilionoideae).
Jean Hanson/ILRI

2 Dehiscent legume pods with seeds.
Jean Hanson/ILRI

3 Weeding forage legume regeneration plots.
Alexandra Jorge/ILRI

4 Harvesting Leucaena seeds from individual plants into separate bags.
Jean Hanson/ILRI

5 Hand cleaning seeds during regeneration.
Alexandra Jorge/ILRI

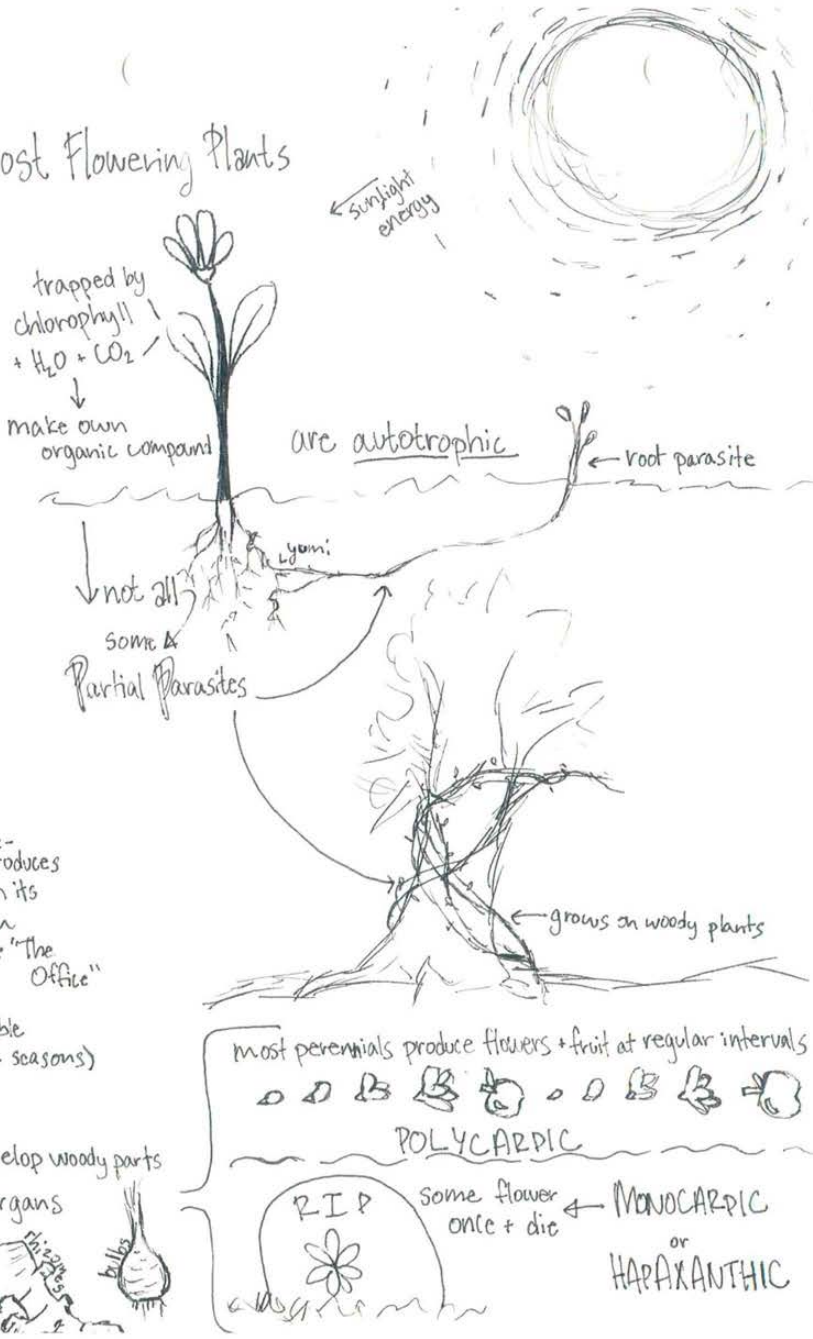
6 Transferring germinated seeds of Vigna unguiculata into seedling trays to raise seedlings for the field.
Alexandra Jorge/ILRI

Plant Identification

Life Forms + Growth Forms

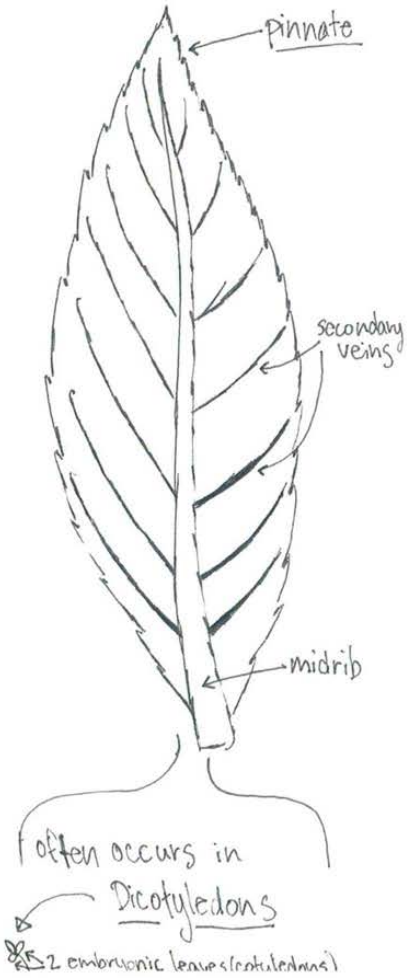
- length of time a plant lives

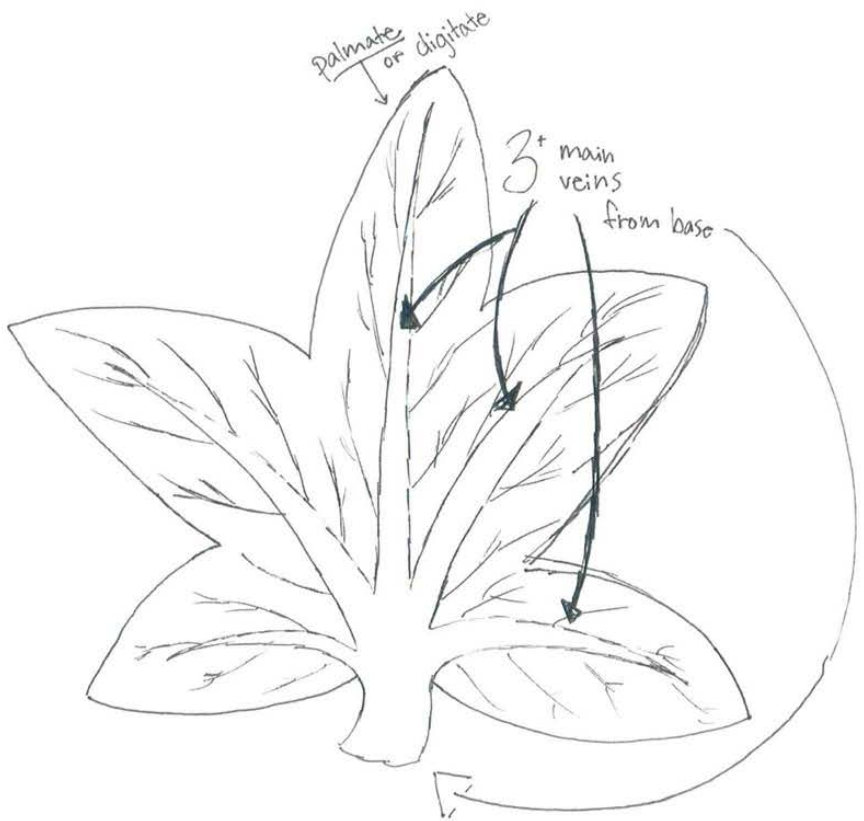
Most Flowering Plants

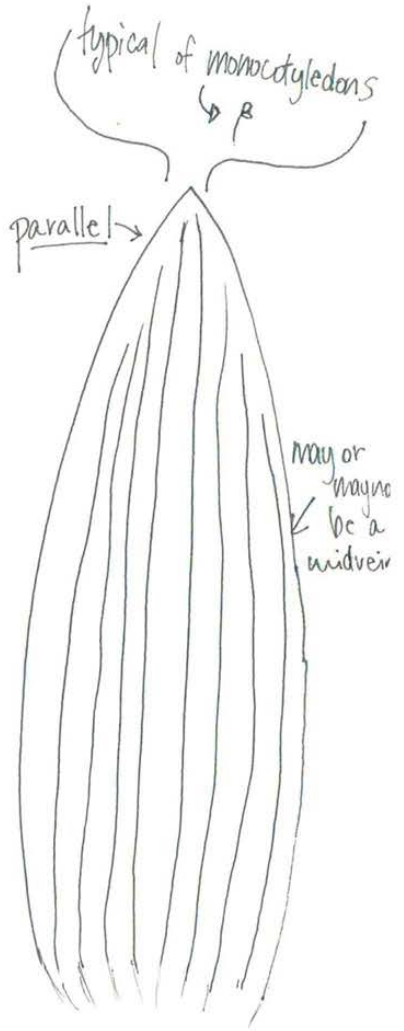


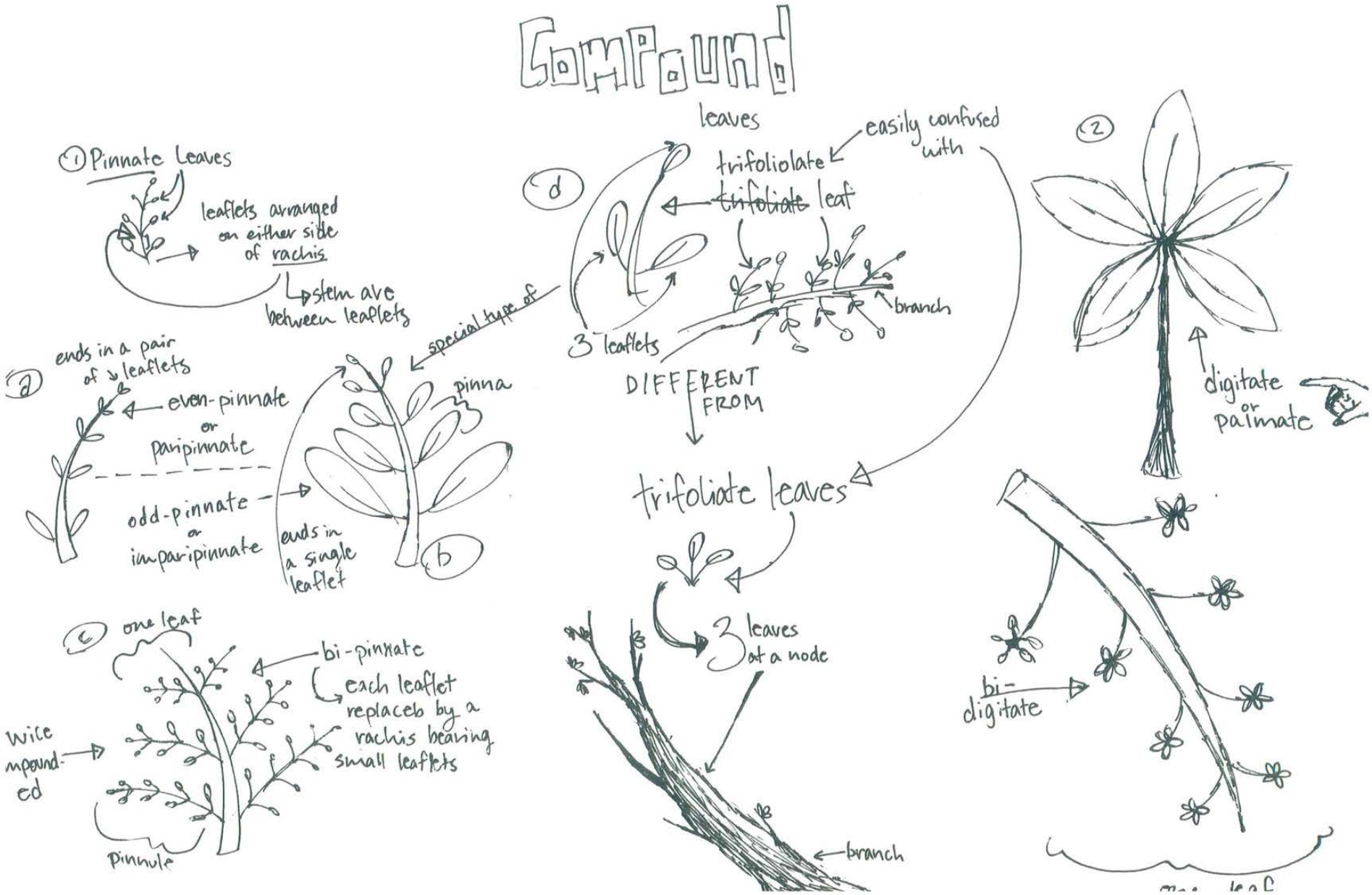
Simple Leaves

-veination pattern

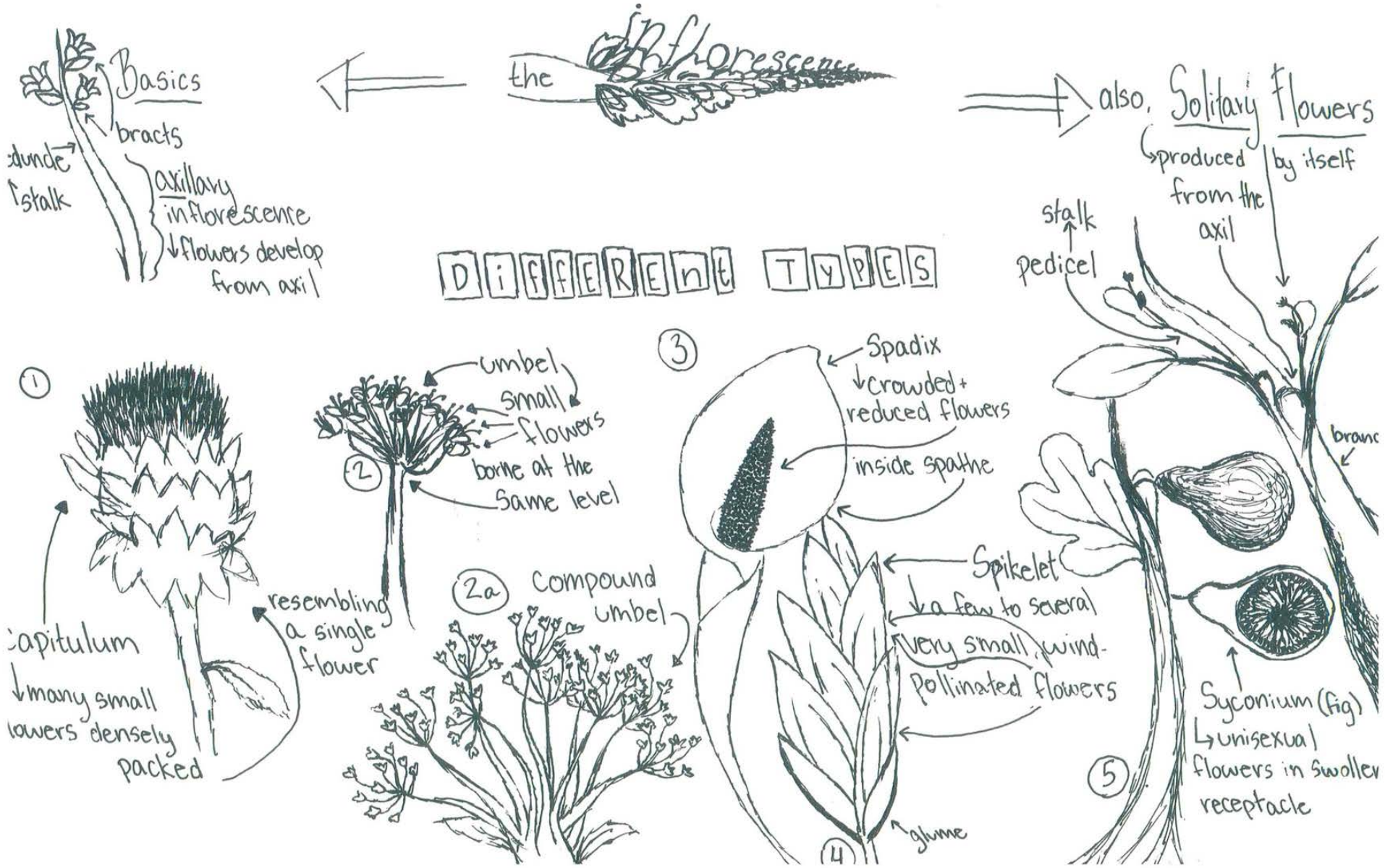






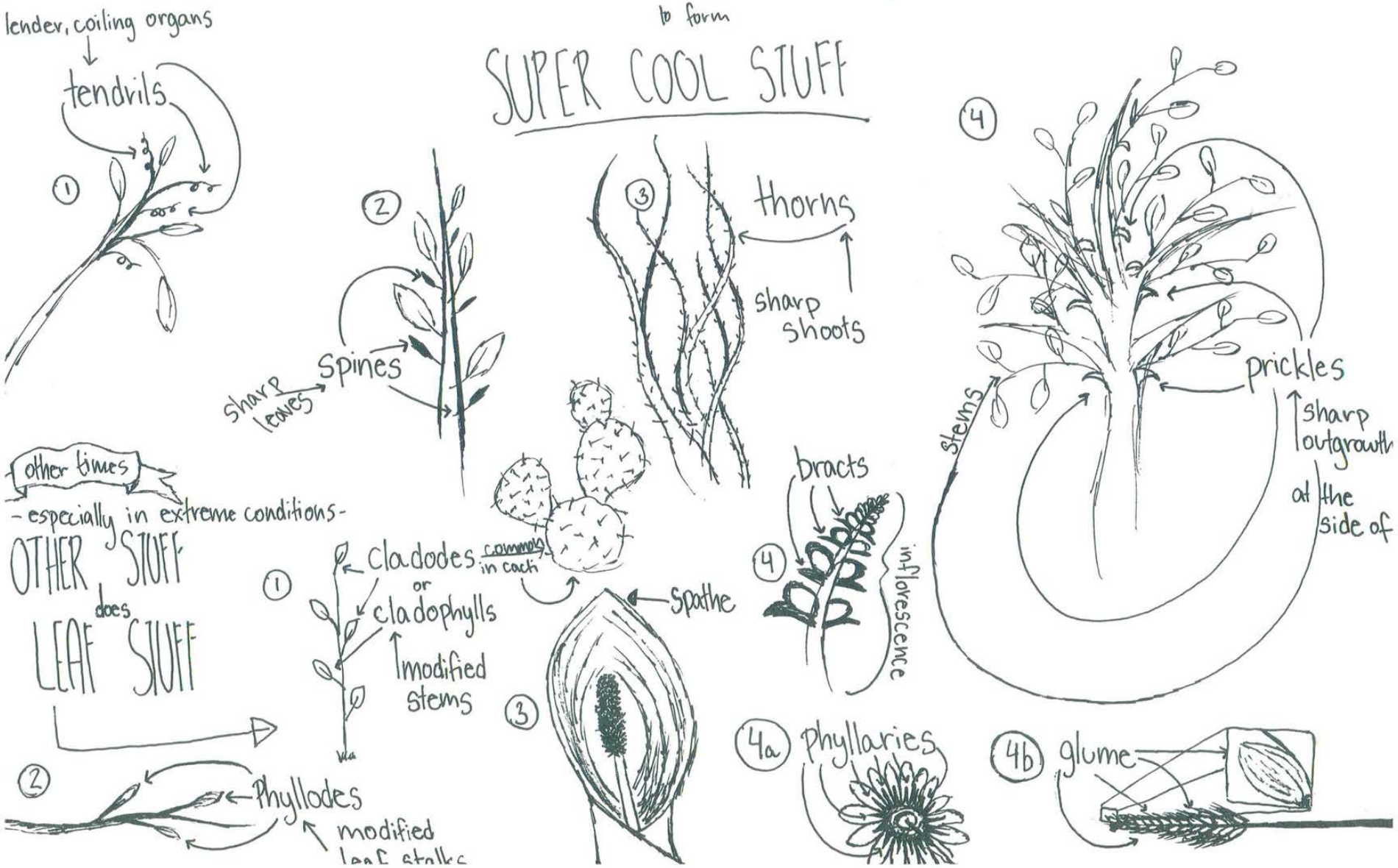

Compound
leaves
easily confused with
trifoliolate
trifoliate leaf
branch
3 leaflets
DIFFERENT FROM
trifoliate leaves
3 leaves at a node
branch
(1) Pinnate Leaves
leaflets arranged on either side of rachis
stem axe between leaflets
(2)
digitate or palmate
bi-digitate
(a)
ends in a pair of leaflets
even-pinnate or paripinnate
odd-pinnate or imparipinnate
special type of
pinna
ends in a single leaflet
(b)
(c)
one leaf
bi-pinnate
each leaflet replaced by a rachis bearing small leaflets
wice mpound-ed
Pinnule
(d)

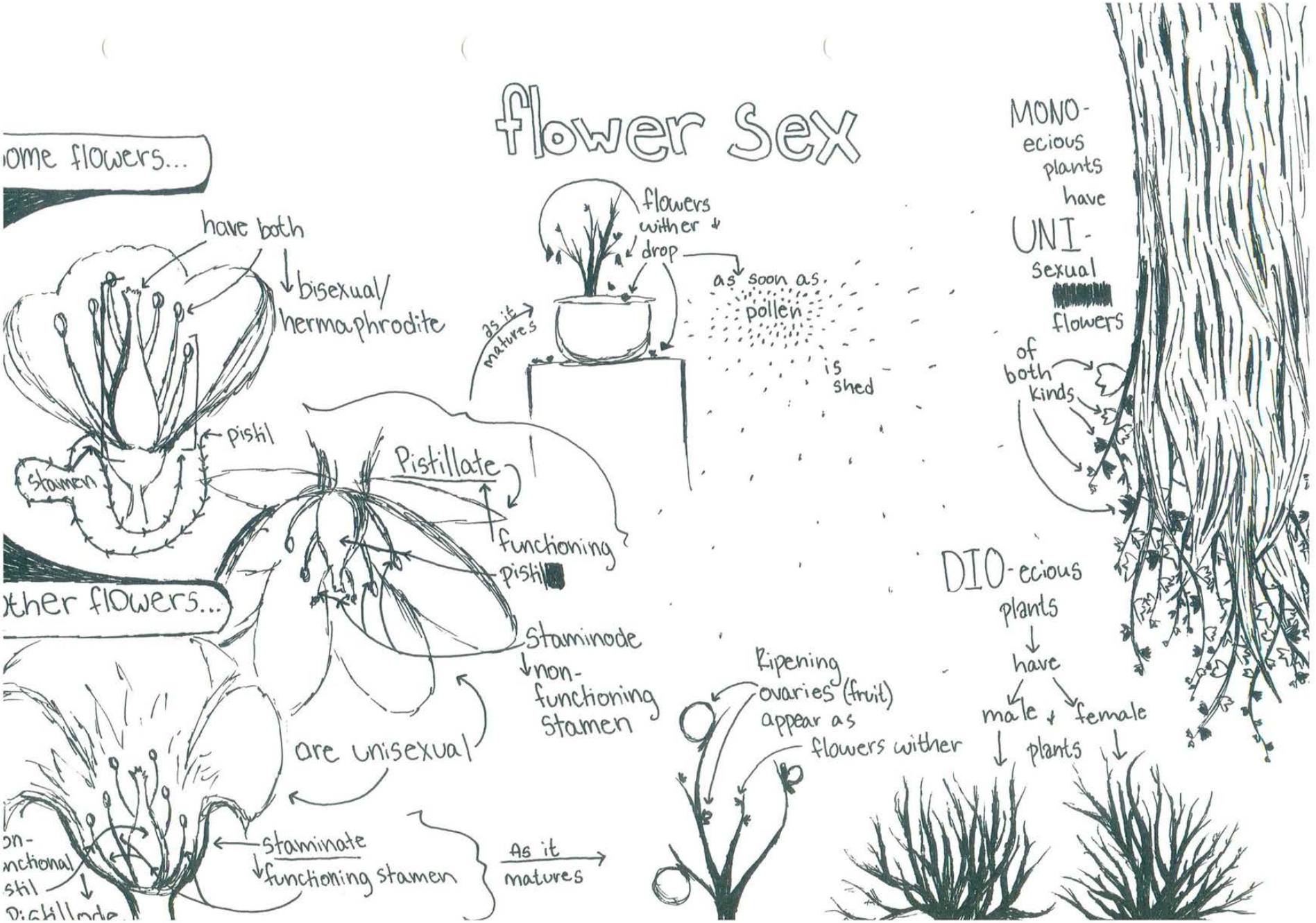
IN SOME
leaves:
each leaflet
has a pair of
stipels
blade/
lamina
sheath
In sessile leaves,
the base can
form a
attached directly
at base
ochrea
ligule
interpetiolar
stipule
SOMETIMES...
Stipules fuse
stipular
sheath
(closed)
stipular sheath
(open)
small flattened
appendages
stipules
Some
are
tightly attached

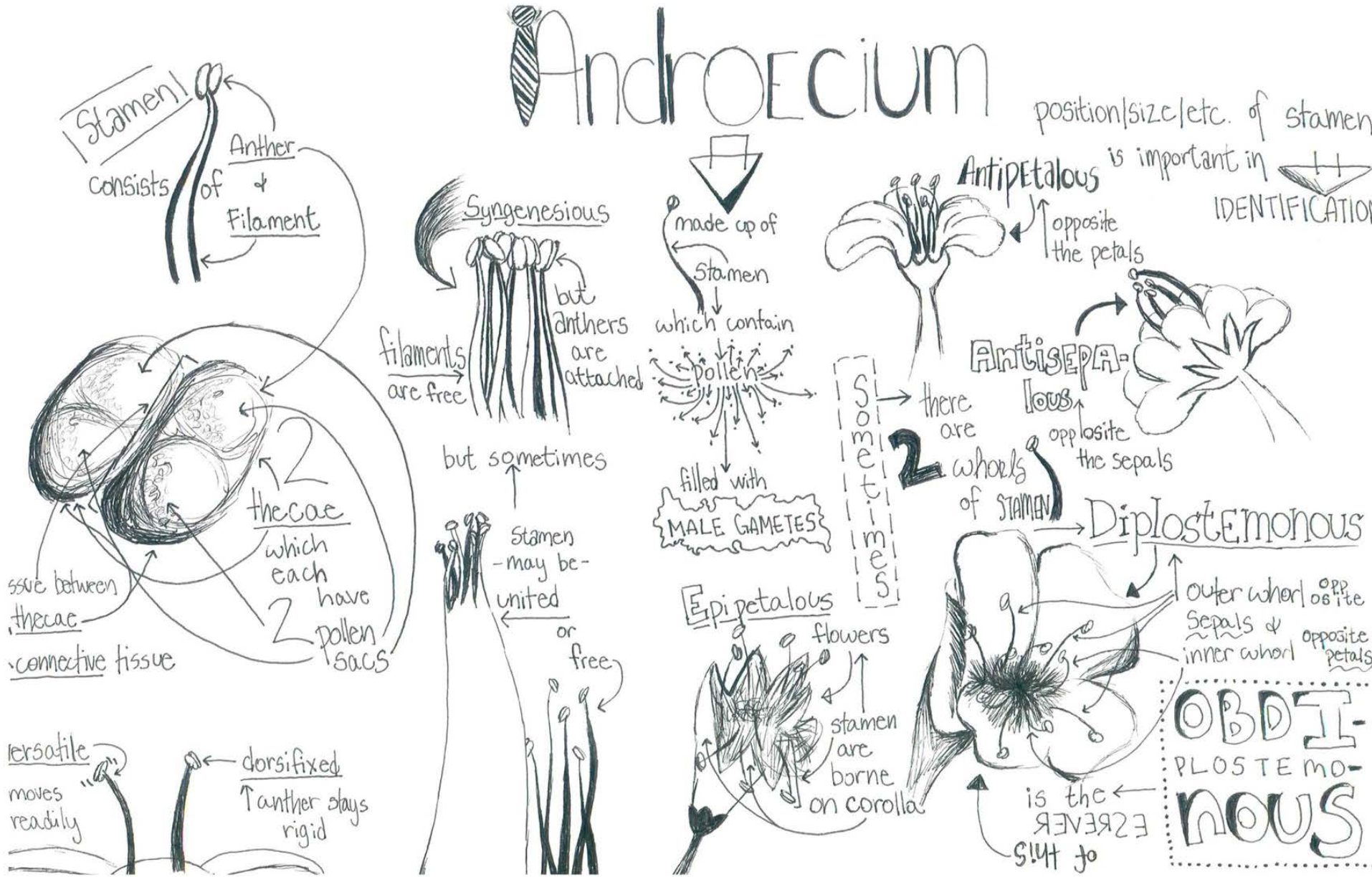

Basics
bracts
stalk
axillary inflorescense
↓flowers develop from axil
the inflorescence
also, Solitary flowers
↳produced from the axil
by itself
DIFFERENT TYPES
stalk
pedicel
1
Capitulum
↓many small flowers densely packed
resembling a single flower
2
umbel
small flowers borne at the same level
2a
Compound umbel
3
Spadix
↓crowded + reduced flowers
inside spathe
Spikelet
↓a few to several very small, wind-pollinated flowers
4
glume
5
Syconium (fig)
↳unisexual flowers in swollen receptacle

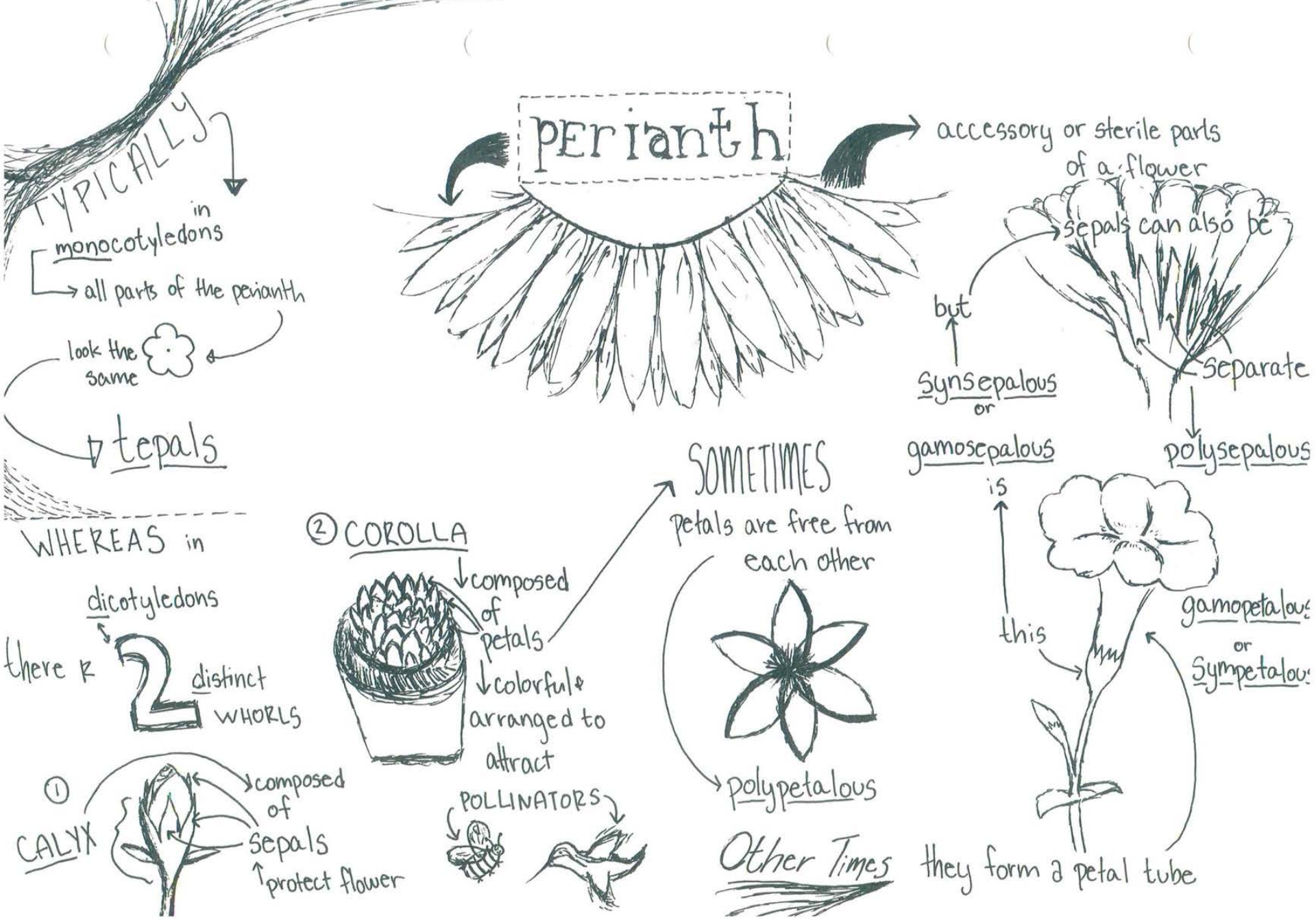
stems, leaves, leaflets + stipules can modify to form

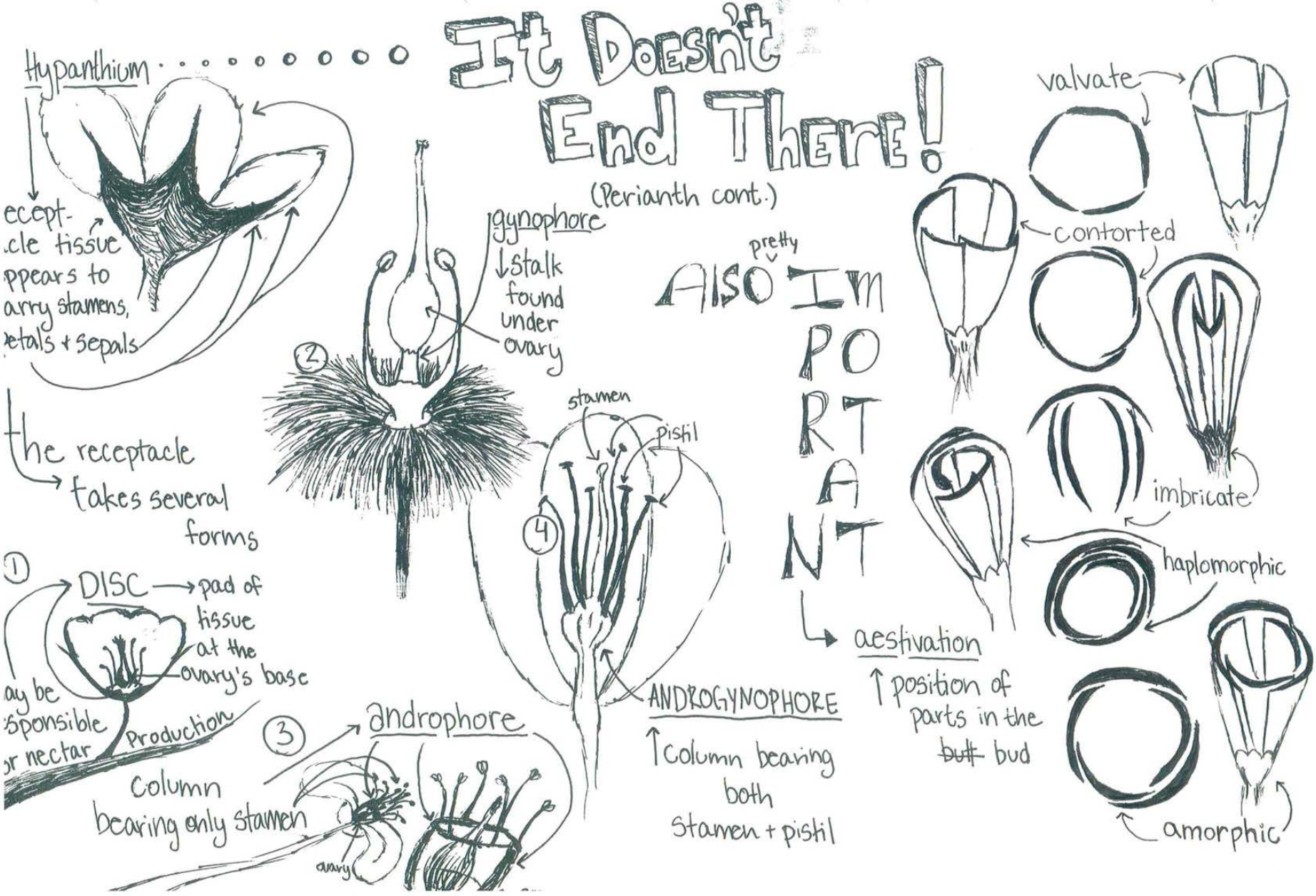
SUPER COOL STUFF




flower Sex
ome flowers...
have both
bisexual/
hermaphrodite
pistil
stamen
Pistillate
functioning
pistil
ther flowers...
Staminode
non-
functioning
stamen
are unisexual
on-
nctional
stil
staminate
functioning stamen
As it
matures
flowers
wither
drop
as soon as
pollen
is
shed
as it
matures
Ripening
ovaries (fruit)
appear as
flowers wither
MONO-
ecious
plants
have
UNI-
sexual
flowers
of
both
kinds
DIO-ecious
plants
have
male
female
plants


Androecium
position/size/etc. of stamen is important in IDENTIFICATION
Stamen
consists of
Anther
&
Filament
2 thecae which each have 2 pollen sacs
tissue between thecae
connective tissue
versatile
moves readily
dorsifixed
↑anther stays rigid
Syngenesious
filaments are free
but anthers are attached
but sometimes
Stamen -may be- united or free
made up of
Stamen
which contain
pollen
filled with MALE GAMETES
Epipetalous
flowers
stamen are borne on corolla
Sometimes
there are 2 whorls of STAMEN
Antipetalous
opposite the petals
Antisepalous
opposite the sepals
Diplostemonous
outer whorl opposite Sepals & inner whorl opposite petals
is the EVERE shut to
OBDIPLOSTEMONOUS


perianth
accessory or sterile parts of a flower
TYPICALLY in monocotyledons
all parts of the perianth look the same
tepals
WHEREAS in dicotyledons
there R 2 distinct WHORLS
① CALYX
composed of sepals
protect flower
② COROLLA
composed of petals
colorful & arranged to attract POLLINATORS
SOMETIMES petals are free from each other
polypetalous
Other Times they form a petal tube
sepals can also be
but
Synsepalous or gamosepalous
separate
polysepalous
this is
gamopetalous or Sympetalous


It Doesn't End There!
(Perianth cont.)
Hypanthium
ecept-
.cle tissue
ppears to
arry stamens,
etals + sepals
the receptacle
takes several
forms
1
DISC → pad of tissue at the ovary's base
ay be
sponsible
r nectar
Production
2
gynophore
↓stalk found under ovary
3
androphore
column bearing only stamen
ovary
4
stamen
pistil
ANDROGYNOPHORE
↑column bearing both stamen + pistil
ALSO pretty IMPORTANT
aestivation
↑position of parts in the ~~bud~~ bud
valvate
contorted
imbricate
haplomorphic
amorphic


Scarification
of seeds
favorable
to ensure
Germination;
SEEDS
often Remain
DOORMAT
WELCOME
DORMANT
this keeps them safe during
SNOW
DROUGHT
snow man
tumbleweed
clouds
AND
ACID RAIN
(maybe)
(probs not)
So
SEEDS
should be
-SCARIFIED-
SEVERAL METHODS
1
SOAK
2
file a
small hole
3
hot
water
4
ACID
5
Digestive System
BUT
this makes
SEEDS
hard to plant
water
nothing
gets
WHY?
SCARIFIED
seeds
40-
50%

PAGE: 3 ILRI GERMPLASM DATA

Passport data:

Accession number: 10898
Genus and species: Trifolium ambiguum

Cultivar: Treeline
Country of collection: New Zealand
Longevity: Perennial
Plant type: Legume
Screening type: Commercial
Growth zone: Temperate

Other accession numbers: CPI-75965

PAGE: 5 ILRI GERMPLASM DATA

Passport data:

Accession number: 20991
Genus and species: Indigofera wightii

Date of collection: / /
Country of collection: VNM
Exact site of collection: W.OF BAN-ME-THUOT,CHEO REO REGION
Altitude: 300m
Annual rainfall: 1200mm
Temperature: 0°C
Aspect: 0 Degrees
Slope: 0%
Parent rock: Quartzites
Soil drainage: Freely draining
Habitat: Woodland/forest margin
Plant height: 0cm
Plant spread: 0cm
Plant type: Browse
Screening type: Experimental
Comments: Clear forest on sndstn. schist

3.4.1 Germination of pollen grains in sucrose

Sucrose concentrations 5%, 10%, 15%, 20%, 25%, and 30% were tested. Each concentration was made by dissolving the sugar in calcium phosphate buffer (0.015M and pH 5.9) containing Boric acid (0.01%). From 3 randomly selected trees, 3 flower buds of different size (17-24 mm) were picked and the size of each bud measured. Then each bud was opened using a fine forceps and few pollen grains were picked and placed on 2 drops of each sucrose concentration on a microscopic glass slide. The pollen grains were uniformly dispersed in the drops and incubated in an incubator at 30 C and 90% RH for 3 hours. The number of germinating pollen grains was counted using an Olympus microscope under X100 magnification and 20 field of view for each concentration. A pollen grain was scored as germinated if an entire tube produced is greater than the grain diameter.

Germination of *Indigofera Wightii* pollen in sucrose

Abstract

The viability of pollen in indigofera wightii was examined using the method of germination. Five slides were prepared, then viewed. Four germinated pollen tubes were found. These results indicate the pollen may be viable.

Introduction

In this lab, I investigated one of the possible reasons for the failure of *indigofera wightii* to produce seed. Since the setting of seed requires successful germination, one possibility is that the pollen present is not viable. This would indicate that there are no male gametes coming into contact with ovules, thus, flowers are continuing to wither and die. As there is currently very little known about the *indigofera wightii*, it is essential to explore each possibility in order to eliminate any which are erroneous.

Germination was chosen as the preliminary method for viability testing because it is one of the easiest to conduct. It will show the capability of *indigofera wightii* pollen to reproduce based on the successful germination of pollen.

Materials and Methods

Materials:

Sucrose

Sodium phosphate

Boric acid

pH tester

200 mL beaker

Magnetic stirrer

Methods:

The sucrose solution was mixed together on June 10, 2014. We chose a medium concentration of 20%. The first step this required was to create a calcium phosphate buffer containing .01% boric acid. The tricky part in this step is achieving the correct pH level. This is important, because otherwise the solution will not act as a buffer. Thus, it was necessary to add several drops of acids, and consequently bases, to even it out to the goal pH. The next obstacle laid in ensuring the concentration of sucrose was correct, and that it would dissolve. We chose a concentration of 20%, because it is a concentration in the middle of those tested. The dissolution of the sucrose, however, proved to be difficult, so we resorted to producing a lower volume of the solution.

On June 18, 2014, I conducted a test to examine the pollen's viability, wherein I brushed many pollen grains onto a slide of the sucrose solution. Each slide, of which there were four, contained a few drops of the sucrose solution and the pollen from one flower. After covering with a coverslip, the slide was placed on a heating table at 9 am and observed at 1:30 pm, to allow for germination.

Results/Conclusion

I observed pollen grains all over the slide- not a surprise as the *indigofera wightii* produces a plethora of pollen. There were also several anthers that had made it onto the slides, as they fell off the filaments quite easily. They appeared to have small, thin protrusions that end in points, but I believe this to be the matter which attaches them to the filaments. The four slides required more than three hours of observation, during which I found only 4 germinated pollen grains, out of hundreds. These grains had definitely germinated, however, with long, clear tubes appearing emerging from the side. One was extremely long; at least five times the size of its pollen grain. The next morning, between 9 and 10 am, I observed three pollen grains which had germinated, but there is no way of knowing whether they were the same as had been found the night before. Regardless, 4/400 is not an encouraging ratio, indicating that only a very slight percentage of pollen is viable.

Stain Solution

The final stain solution we used was prepared by adding the following constituents in the order given below and stored in the dark.
10 mL 95% alcohol
1 mL Malachite green (1% solution in 95% alcohol)
50 mL Distilled water
25 mL Glycerol
5 mL Acid fuchsin (1% solution in water)
0.5 mL Orange G (1% solution in water)
4 mL Glacial acetic acid
Add distilled water (4.5 mL) to a total of 100 mL.

Staining

Following at least two hours of fixation, the bud can be placed on a microscope slide and the fixative's liquid was thoroughly and carefully dried from the plant material with absorbent paper. Proper safety gloves should be worn to avoid the risk of chloroform from being absorbed through the skin. Apply 2-4 drops of the stain solution before the sample completely dries. If flower buds have been collected instead of free anthers, the buds should be dissected to release the anthers and pollen. Under a dissecting microscope the leftover plant debris can be carefully removed. To save stain solution, samples can be dissected prior to putting individual anthers into stain. Some anthers, such as those of Magnolias, are too large to be viewed intact and must be dissected further.
Once the sample is in the stain, slowly heat the slide over an alcohol burner in a fume hood until the stain solution is near boiling (~30 seconds). A more moderate rate of heating allows better penetration of the dye into the cellulose and protoplasm of the pollen. Extremely high temperatures resulting in smoking or bubbling of the stain can burn the dye and the sample. Heating can be adjusted by briefly moving the slide in and out of the flame. To ensure stain has been completely absorbed into the pollen grains, 10 to 15 minutes should be allowed for some species such as *Lonicera tatarica*, *Ginkgo biloba*, *Pinus resinosa* and *Rhododendron mucronulatum*.

http://www.pagepress.org/journals/index.php/pb/article/view/pb.2010.e13/2315

Staining of *Indigofera Wightii* pollen

Abstract

The viability of pollen in *indigofera wightii* was examined using the above stain solution. Four slides of the solution were prepared. 63.7% of the pollen was apparently stained. These results indicate the pollen is more than likely viable.

Introduction

In this lab, I investigated one of the possible reasons for the failure of *indigofera wightii* to produce seed. Since the setting of seed requires successful germination, one possibility is that the pollen present is not viable. This would indicate that there are no male gametes successfully coming into contact with ovules, thus, flowers are continuing to wither and die. As there is currently very little known about the *indigofera wightii*, it is essential to explore each possibility in order to eliminate any which are erroneous.

This lab provides a clearer look into the viability of pollen. Previously, I had tested it with a germination test, but the results of that test were inconclusive. The stain test is a more distinct indicator as it is obvious which grains are stained and which aren't. This allows for a proportion to be obtained and generalized.

Materials and Methods

Materials:

Lab coat

Plastic gloves

Scale

Measuring spoon

Plastic dish

Bottles w/lids

10 mL graduated cylinder

50 mL graduated cylinder

200 mL beaker

Stirring stick

Labels and pen

Microscope slides

Microscope slide covers

Heat source

Methods:

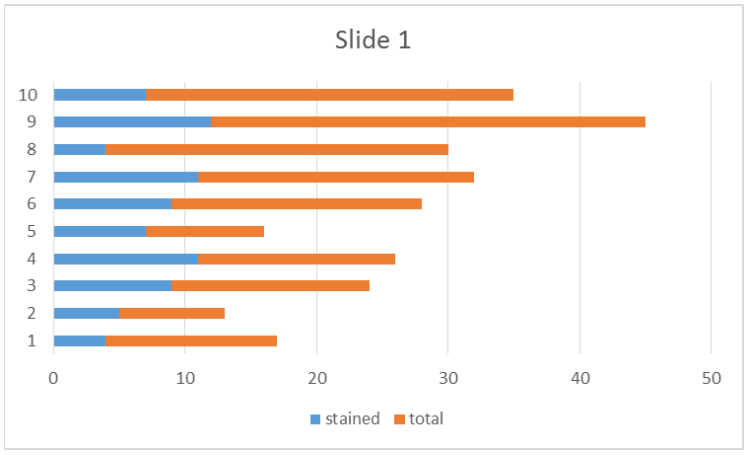
The experiment was conducted at the ILRI Forage Diversity labs. Since the chemicals we were dealing with were acidic, staining, and harmful on contact, we took precaution by wearing gloves and lab coats. Our first step was composing 10 mL of 95% alcohol. Since we had only 99% alcohol on hand, we took 9.5 mL of that and added in .5 mL of ddH_2O. Next, we put together the other solutions that would be combined later. For malachite green, this required also mixing up some more 95% alcohol. Once this was done we measured out .1 g of brilliant green powder, as we had no malachite green on hand and brilliant green can be used as a substitute. Then we mixed that with 10 mL of 95% alcohol to create a 1% solution (of which only 1 mL was eventually used). Similar procedures were followed using acid fuchsin and orange g, of which we composed 5 mL solution of each with ddH_2O, this time measuring out .05 g of the powder. These were all mixed by pouring into small bottles with lids and shaking until homogeneous. Once these were ready to go, all components were mixed together in order using a 200 mL beaker. As fumes from glacial acetic acid are highly harmful, it is necessary to use a fume hood while adding it, to prevent the inhalation of toxic vapors.

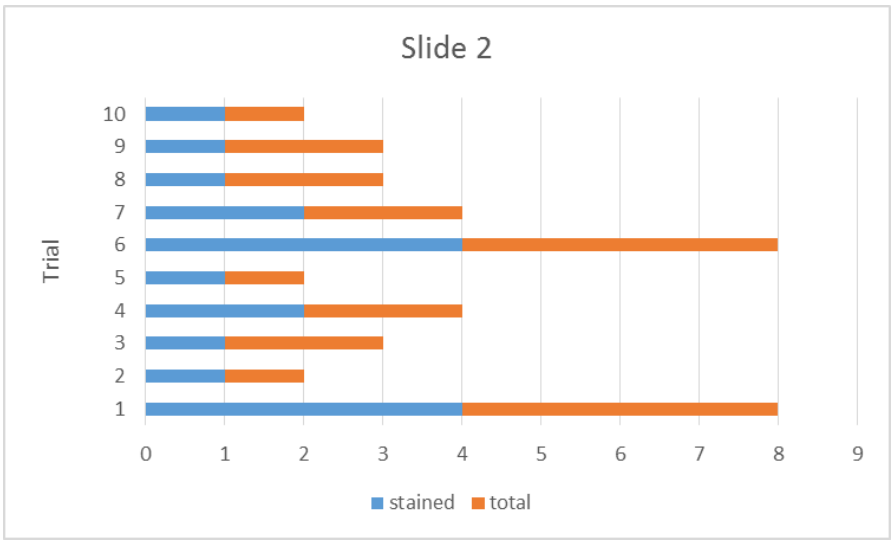
After mixing the solution, I placed 1-2 drops onto the end of a microscope slide and brushed pollen grains into that. Our heat source of choice was a spirit lamp, which is a small glass bottle containing a thin layer of alcohol with a thick wick extending from the alcohol to peek out of a hole in the aluminum foil covering. Then a match is used to light this tip, allowing for an easy, portable, fast flame. After about 30 seconds of exposure the stain sample would boil and be removed from the flame. Following this, a cover slip was placed over the sample and viewed after 10-15 minutes.

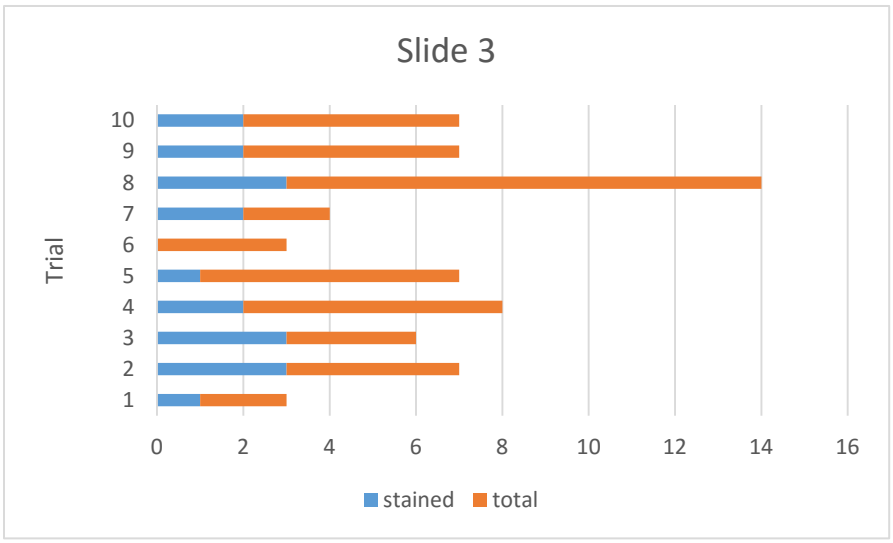
Results/Conclusion

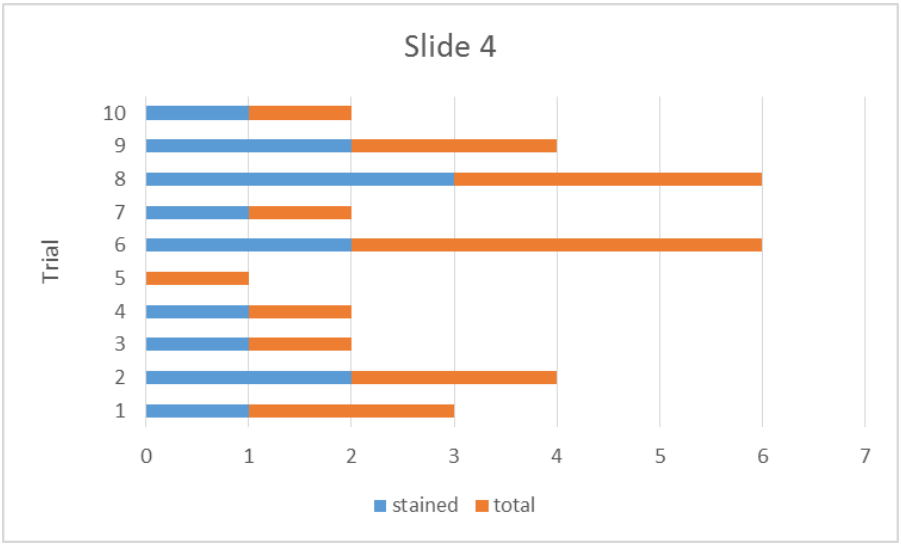
Under an Olympus microscope at 40x magnification, the pollen grains were viewed. The stained pollen grains were obviously different, a stark contrast to those which were unstained. In order to obtain a proportion without counting each individual pollen grain, I noted how many grains were stained out of the total in each window available through the microscope lenses. The resulting proportion was 174/273, or 67.3%.

The below charts show the results of the stain test for each slide:









This test indicates that over 2/3 of pollen are more likely than not viable in the average indigofera wightii flower. This would suggest that there should be an issue in germination and seed production, as far as pollen viability is concerned, only 32.7% of the time.

Bedinger Lab Tomato: Pollen Staining Protocols

(September 22, 2010)

Emasculation

1. First, emasculate -1 stage tomato flowers (closed to slightly separating pale yellow petals. This is the stage of flower right before Bud Break, before any pollen is released from the anthers. The idea is that you are emasculating a flower that if left alone would open after 24hrs) by removing the anthers.

2. Mark the flowers by labeling with the female accession #, what it will be used for, and the date of pollination. Wait 24hrs.

3. Pollination is performed the next day by touching the stigma onto a surface (e.g. a 1.5 ml microfuge tube lid, or flower anthers) covered with pollen. Note: Pollen can be collected various ways, we mimic the vibration of bumble bee by using a hand-held tooth polisher to collect the pollen in a microfuge tube.

4. Pollinations are allowed to progress for 24 hours, unless specified otherwise (pollen tubes tend to be about 40% of the way down the style at 6 hours in a compatible/congruent pollination). Field flowers should be protected from pollinators by covering the inflorescence.

Fixing Pistils

1. After the desired amount of time post pollination, the remaining sepals, and petals are removed from around the pistil.

2. Using a scalpel or razor blade the pistil is cut at the base of the ovary just above the pedicel.

3. The excised pistil is placed in a 1.5 ml microfuge tube containing 0.5 ml (enough to completely submerge pistils) 3:1 95% EtOH: glacial acetic acid and left overnight or indefinitely.

Softening Pistils

1. Fix is removed by piptetting, and pistils are then submerged in 0.5 ml 5 M NaOH softening solution for 24 hours. Note: the tissue becomes extremely fragile and can be easily damaged after this stage. Also, the pistils tend to float right after adding NaOH so it good to gently shake the tube after a couple of hours and get the pistil to sink to the bottom.

Staining Pistils

1. After 24 hours the 5 M NaOH is carefully removed by pipetting (taking care not to disturb the pistil) and the pistil is gently washed 3-5 times with 0.5 ml ddH20 each time.

2. After the last wash the ddH2O is removed and replaced with 0.2 ml 0.001mg/ml ABF (Aniline Blue Fluorochrome) in 0.1M K2HPO4 pH 10 buffer.

a. Aniline Blue Fluorochrome was obtained from Biosupplies Australia (http://www.biosupplies.com.au). We prep a stock solution of 0.1mg/ml in ddH2O from the dry ABF they send. This is stored at +4 C. A further dilution of the stock solution to make a working stock solution is done using a 1:20 dilution of the stock solution into 0.1M K2HPO4. (In the past we have also used a 100X solution works best for us but you should try different amount that best work for your system).

3. Once the stain is added to the tubes, samples are immediately placed in the dark and allowed to sit for about 24hrs for best staining.

Mounting/Viewing Pistils

1. After incubation in ABF for 24 hours, pistils are removed and placed in a drop of 50% Glycerin on a microscope slide and covered with a cover slip.

2. View with a standard fluorescence microscope capable of exciting with a UV light source and DAPI emission filters to view the fluorescent signal from the tissue.

3. After viewing is complete, take nail polish and seal the edge of each cover slip. This stores the slide material so that we may return to it later.

www.irbtomato.org/Aniline_Blue_Staining_Protocol.pdf

Indigofera wightii in Analine Blue Fluorochrome

Abstract

The viability of pollen in *indigofera wightii* was examined using Analine Blue Fluorochrome stain. Five pistils were prepared and stained, then viewed. Germinated pollen tubes were found to be fluorescing on the stigma. These results indicate the pollen is viable.

Introduction

In this lab, I investigated one of the possible reasons for the failure of *indigofera wightii* to produce seed. Since the setting of seed requires successful germination, one possibility is that the pollen present is not viable. This would indicate that there are no male gametes coming into contact with ovules, thus, flowers are continuing to wither and die. As there is currently very little known about the *indigofera wightii*, it is essential to explore each possibility in order to eliminate any which are erroneous.

So far, the *indigofera wightii* has refused to produce seeds, even in response to hand-pollination attempts. Furthermore, first two tests in which nearly contradictory results were displayed, it was necessary for a final one to break the tie.

Materials and Methods

Materials

Lab coat

Plastic gloves

Petri dish

Scalpel

Microfuge containers (5)

Ethyl alcohol

Glacial acetic acid

200 mL beaker

Stirring stick

10 mL graduated cylinder

30 mL graduated cylinder

pipette

NaOH

ddH_2O

Scale

Measuring spoon

Analine Blue Fluorochrome

K_2HPO_4 buffer (pH 10)

Microscope slides

Coverslips

50% glycerin

Methods

The first step in AFB staining is pollination of the stigma. For my trials, I collected eight flowers on July 7, 2014, and emasculated them under a microscope, gathering the pollen a petri dish. The microscope was necessary because of the miniscule size of the *indigofera wightii* flowers- making emasculation without damaging the other parts of the flower nearly impossible. Then, I took 5 of the flowers with only intact pistils and brushed the stigma onto pollen grains. They were easily caught by the stigma as it was receptive: shiny and gel-like. These pistils, still attached to the receptacle and sepals, were each placed in separate microfuge tubes to germinate for 24 hours.

The next day, July 8, I preserved the pistils by placing them in 2 mL of EtOH:glacial acetic acid fixative. This solution was composed by first combining 28.5 mL of EtOH with 1.5 mL of ddH_2O in order to achieve 95% EtOH. Then, to reach a 3:1 ratio, 10 mL of glacial acetic acid was mixed in. It is important to utilize a fume hood when performing this step to ensure none of its toxic fumes are inhaled. Before adding the pistils to the fixative, I cut the pistil free from the remaining perianth, receptacle, and sepals.

After about eight hours, I carefully pipetted out the fixative and added 2 mL of NaOH softener, making sure to rinse the pipette with ddH_2O in between. This was then left overnight. The next day, July 9, I rinsed each pistil cautiously with ddH_2O several times before adding 2 mL of Analine Blue Fluorochrome solution for staining. I then placed the microfuge tubes in a petri dish for protection, which I then put in a drawer, as darkness is necessary for optimal staining.

The next day, July 10, I placed the stained pistils in a few drops of 50% glycerin on separate microscope slides and enclosed them with coverslips. They were then ready for viewing under a UV light on an Olympus microscope at 40x magnification.

Results/Conclusion

I observed that the stigma on each of the sample was highly fluoresced, glowing a bright green under the UV light. There were also visible tubes which ran down the style, but they appeared more like veins than pollen tubes. On the tip of the stigma, there were dozens of short tubes extending from the end of its, looking like miniscule hairs sprouting out. They were also highly fluorescent, with an aura of green radiating from them. There were many ovules present in the style, also fluorescing, but there did not appear to be any pollen inside the style. There were small, round, fluorescent object surrounding the stigma, but there were no apparent nuclei inside, so I remain unsure as to whether they were indeed pollen grains. From this test I can conclude that the pollen is seemingly viable, but for some reason will not germinate.

A repeated trial of this stain resulted in visible pollen tubes formed in the stigma, but not reaching the ovules. This confirms the prior finding, that the pollen is viable.

Red Spider Mite

The red spider mite is actually a member of the *arachnidia* family, meaning that it can't truly be classified as an insect. They are very small, however, between .3 and .5 mm. They are an oval shape and reddish brown, with four pairs of legs. The life cycle of the spider mite is extremely short, which allows for a rapid infestation. After only 4-7 days, the clear, spherical eggs break open to reveal 6-legged, flesh-colored larvae. There are two adolescent stages- protonymph and deutonymph, which each last 3 days. After this, the adults may reproduce from 10-30 days, laying 10 eggs a day. This means that in one season, 6-8 overlapping generations can be produced. The spider mite feeds by piercing leaf cells and sucking out any fluids. They are versatile in their choice of host plant- there is a wide variety and number of plants which can be home for the parasite. As the population grows, they will spin webbing to surround the plant, which serves a dual purpose of a protective layer and as a means for transportation. Spider mites will often congregate at the top of a plant in the hopes that they will be able to travel by wind or animal.

Spider mites can first by evidenced by stippling that appears on leaves. These are yellow and white spots that are caused by the sap are drained and the cells begin to bruise. Eventually, the leaves will shrivel, turn brown, and drop, as the plant loses all photosynthetic ability. In extreme cases, webbing will cover the plant, but it is possible to catch the mites once stippling is noted. It is necessary to examine the underside of the leaves, as this is where they colonize.

Spider mites thrive in dry weather, as it allows for the evaporation of extra excrement and it kills of many of its natural predators. Therefore, the first line of defense should be consistent and adequate irrigation of plants, even applying water to walkways and other dusty areas. There are many pesticides that can be applied, both chemical and non-chemical, but caution and consideration must be taken to ensure that the pest still remains, as treatment otherwise will adversely affect the plant. Furthermore, some insecticides actually favor spider mites by killing off natural predators. Others stimulate mite reproduction and raise the nitrogen levels, which further benefits the parasite.

Black Fly Aphid

The Latin name for black fly aphids is *aphis fabae*, but they are often called black bean aphids as their dark, oval bodies resemble miniature version of the legume. They also have a quick regeneration period and takeover pace, as nymphs become reproducing adults in one week. They are the most widespread garden pest because they are polyphagous- meaning they are able to feed off many types of plants. They do this by sucking out sweet plant juices through their miniscule, straw-like beaks. At ¼ inch lengthwise, they are easily seen by the naked eye, aided by the fact that they tend to inhabit flowers, shoots, and young leaves, placing them at the top of the plant.

Another factor in their rapid infestation is the rate and type of reproduction. Each female can produce 5-8 offspring/day for 30 days, or about 50-100 young in her lifetime. In addition, aphids reproduce by parthenogenesis. This is a form of incomplete sexual reproduction wherein no male is needed. Instead, the egg is stimulated, and then the genetic material is doubled and divided, resulting in two sets of identical chromosomes, both from the mother. Thus, each offspring is a replica of the mother. In warmer climates, such as Addis Ababa, it is possible for aphids to continue reproducing partheogenetically for years.

In more temperate climates, however, some aphids will develop into males later in the year. This allows for eggs to be fertilized which can overwinter on the plant after being laid. Occasionally, the males who develop will be incomplete, missing a mouth or other body parts. Literally, their only purpose is to fertilize eggs. This seemingly spontaneous development is also seen when there is a need to move to a different plant. In these cases, winged varieties will develop.

The effect of aphids can vary from an annoyance to a something, but they will rarely kill a mature plant. They are attracted by sugar and sweet sap stored inside stems and flower. As they suck it out, they create a secretion called honeydew which can develop into a sooty mold. Their removal of vital fluids will also cause stunted growth and curled leaves. The greatest and most dangerous impact they can have is the transmittal of disease as they move between plants; an infection can occur even in very small populations. Alternately, however, they can be beneficial on adult trees, where the honeydew attracts good insects and provides a food source.

The honeydew secreted always draws ants to the plant. Since they are much larger than aphids, they can be an easy and immediate indicator that there is an aphid infestation. Ants have a symbiotic relationship with aphids wherein they eat the honeydew. In return, they 'tend' aphids, protect them from predators, and sometimes even carry them around the plant or to a new host. Thus, one means of control is to prevent access to the aphids. This can be achieved by placing a ring of duct tape or other sticky material around the base of the plant.

There are many other means- chemical, non-chemical, biological- of protection and control. There are a variety of insecticides available, but care must be taken to ensure that is not systemic, which will be engulfed by the plant. Furthermore, most insecticides require contact to kill, so it is important to coat the entire plant as aphids often colonize the underside of leaves. This is why catching the infestation early is essential, because the curling leaves will offer the aphids protection.

On the infected plants at ILRI, we will use heavy jets of water and isopropyl alcohol on cotton swabs. Other non-chemical options include introduction of the aphids' natural predators, such as Ladybirds, lady beetles, lacewig larvae, soldier beetles, and syrphid fly larvae. When practical, it is also possible to simply squash the aphids. Addtionlly, in thecase of a localized infestation, removing that part of the plant may be the best option. The best choice is of course prevention, meaning vigilant monitoring for warning signs. In addition, marigolds, if planted nearby, will repel aphids and attract natural predators with its scent.

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Seed Producer Survey

1. Name
2. Gender: Male/ Female Manager/Owner
3. What is your highest education level?
4. How did you get into seed production?
5. Do you have any other businesses?
6. Do you have livestock?
    a. What kinds?
    b. For what?
7. How much land do you use for forage seed production?
8. Do you own the land?
9. Do you use all the land available for forages?
10. How many (i) supervisors and (ii) workers are employed? (i)_____ (ii)_____
11. Why do you grow forage?
12. Have you always grown forage?
13. What types?
14. How do you decide which forage to grow?
15. How do you plant?

16. How do you harvest?

17. How high is your seed yield?

18. What is your best seller?

19. How do you market your seeds?

20. Who do you sell to?

21. What are your strengths in seed production?

22. What areas do you need to develop?

For unions/cooperatives

23. How many seed producers in the union?

24. How many male/female members?

25. When was the union organized?

26. What are the benefits of being in a union?

27. What is the impact on individual seed producers?

28. What are your responsibilities in the union?

Survey Results

Seed Producer	Gender	Education Level
1	Female	4
2	Male	11
3	Male	16 BA business/accounting/finance
4	Both male	18 MSc development planning/ BC plant science
5	Male	16 BS plant science
6	Male	10
7	Male	10
8	Female	12
9	Male	20 PhD Veterinary Medicine

Seed Producer	Livestock	Forage Only	Supervisors:Laborers
1	Dairy cow, oxen, chicken,donkey	No	5:6
2	Dairy cows, donkeys, oxen	Yes	1:3
3	Fattening cows, oxen	No	3:20
4	Dairy cows, oxen	No	2:10
5	No	No	5:121
6	Dairy cows	No	1:20-30
7	Goat, oxen, sheep, dairy & fattening cows	Yes	1:≤30
8	Fattening & dairy cows	No	2:10
9	Fattening cows	Yes	2:10

Seed Producer	Amount of land for forage (ha)	Why start farming?	Why forage?
1	2	Feed livestock	Animals
2	0.5	Livestock feed	Livestock, income
3	3	Market demand	New market
4	1	Interaid France-train farmers, land here	High demand, high interest
5	27	Grew hybrid maize, haricot bean	Past-cattle fattening, now-save, sell, soil conservation, assist community-develop soil fertility
6	2.53	Cattle feed, then tech assistance-how to grow forage	Cattle feed

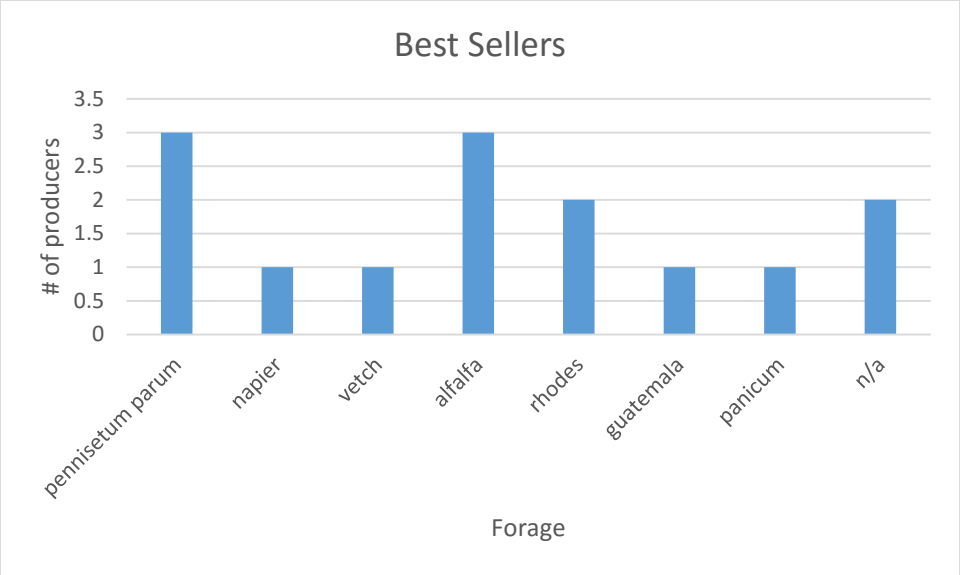
7	7	Used to work for WorldVision-nursery manager-learned about seedlings-began growing forage & trees-farm & provide seeds	Shortage of seed-business, plant matter, hay
8	4	ILRI-this year	Cattle, sell seed
9	5	ILRI invite-took training	Cattle, seed

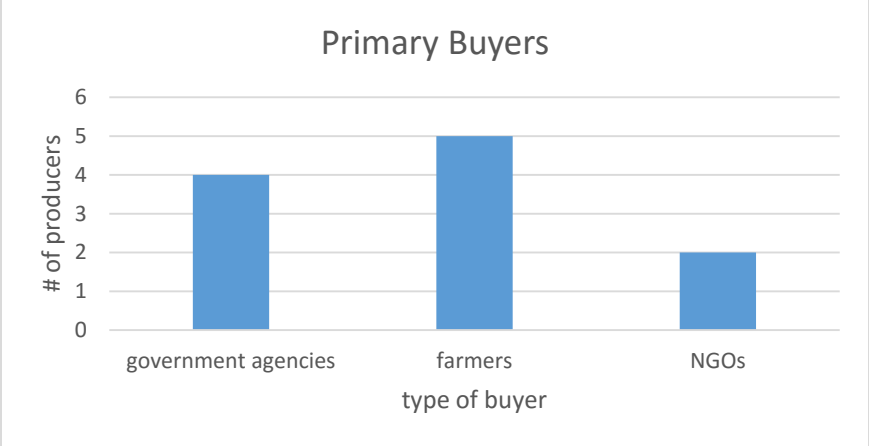
Seed Producer	Always grown forage?	Decide which forage?	How do you plant?
1	Yes	NGO- VOCA advise	Manual labor/plow
2	Few years ago	Best for livestock	Manual labor
3	Yes	Market prices/demand	Oxen/tractor
4	Yes	Environment adapted	Oxen
5	No	Gov't gives it to them	Tractor
6	Yes	Rotation soil protection, feed, sell the best	Manual labor
7	No	Demand	Manual labor
8	No	Environment adapted	Oxen
9	Yes	Environment adapted	Manual labor

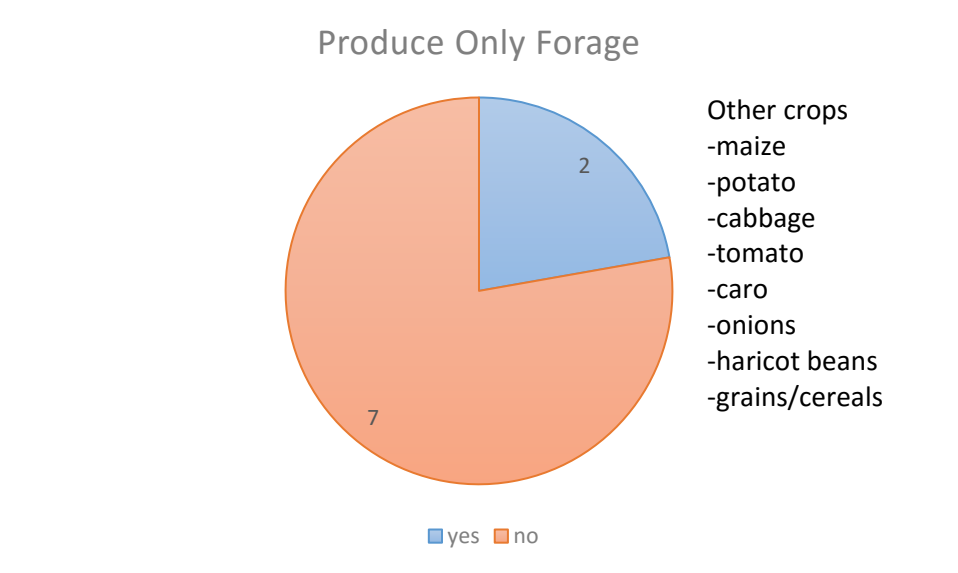
Seed Producer	How do you harvest?	Seed yield (kg/ha)	How do you market seeds?
1	Manual labor	100	Come and buy
2	Manual labor	50	Come and buy
3	Manual labor	n/a	Go to individuals
4	Manual labor	40	Come and buy/gov't encourages to buy here
5	Manual labor	10	Come and buy
6	Manual labor	200	Come and buy
7	Manual labor	100	Gov't NGO advertise
8	Manual labor	n/a	Introduced to buyers
9	Manual labor	n/a	NGO's- ag offices

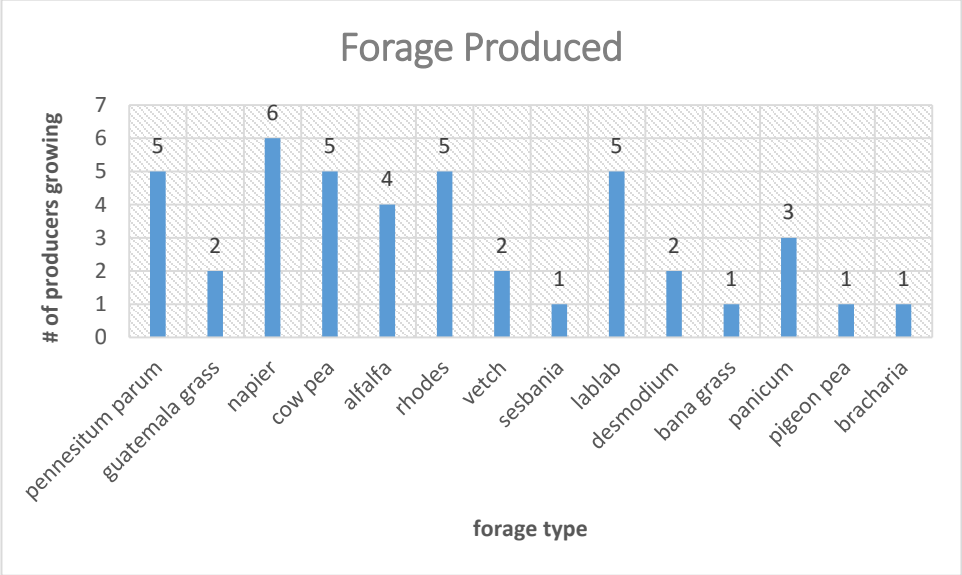
Seed Producer	Strengths	Needs to develop
1	Management, learn from experience, weeding	Cross bred crows-produce more milk to sell because they have the feed

2	Improved forage, yield, other beneficial qualities	More land-more rhodes, alfalfa, others, grow & expand
3	Willingness to work, land qualified people	Harvesting team & packaging, add drip irrigation-water pumps
4	Background in ag, no big supply=large demand, rain fed	Produce quality seed, research & business intensifies, new market, benefits
5	Have a lot of land, good crops, good management	Improve management, improve tech. support with ILRI, diversify with other forages
6	Sells seeds-feeds cows forage=use all of the plant & land serves as demonstration site-people come to learn	Intensively develop & expand
7	Merchant- also has land & multiplies	More land, part of seed-multiply
8	Irrigation system	More land-already in motion
9	Big storehouse, large feedlot	100 ha for grasses

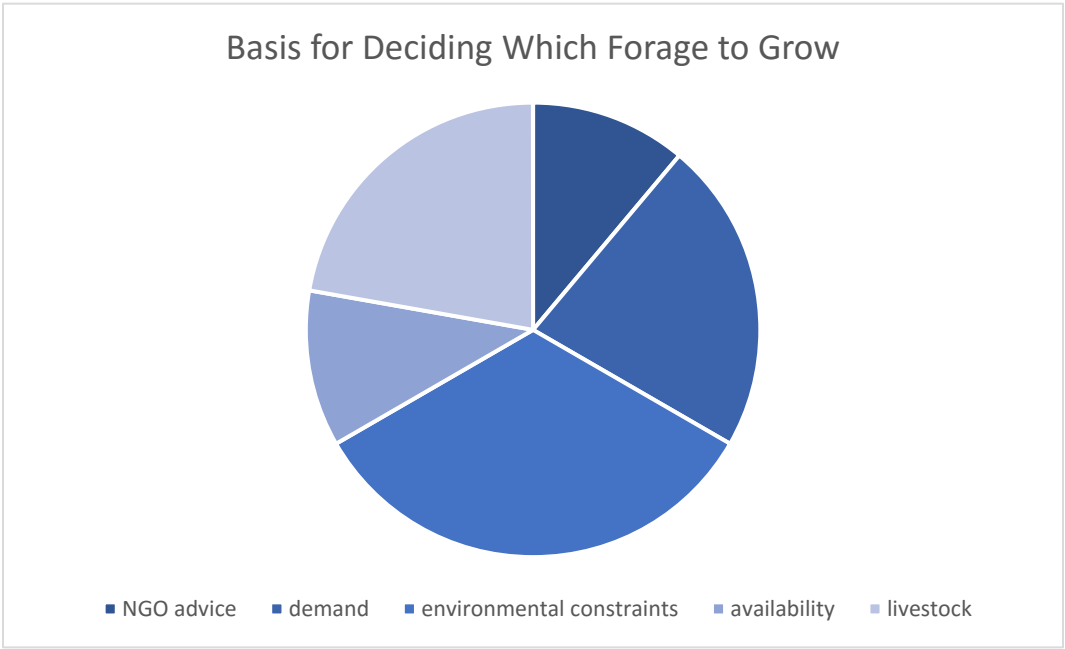

Best Sellers
# of producers
3.5
3
2.5
2
1.5
1
0.5
0
pennisetum parum
napier
vetch
alfalfa
rhodes
guatemala
panicum
n/a
Forage

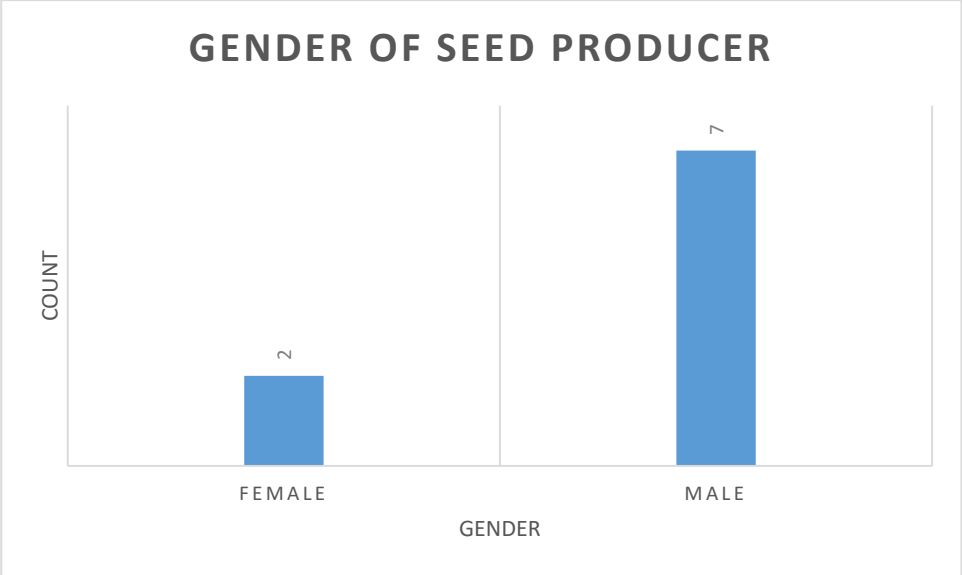

Primary Buyers
# of producers
6
5
4
3
2
1
0
government agencies
farmers
NGOs
type of buyer

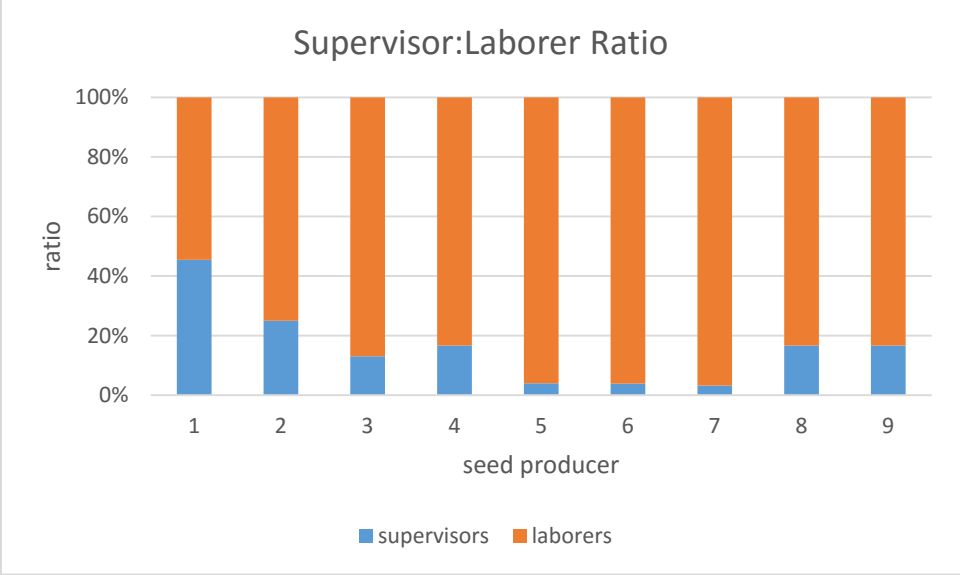

Produce Only Forage
2
7
yes
no
Other crops
-maize
-potato
-cabbage
-tomato
-caro
-onions
-haricot beans
-grains/cereals

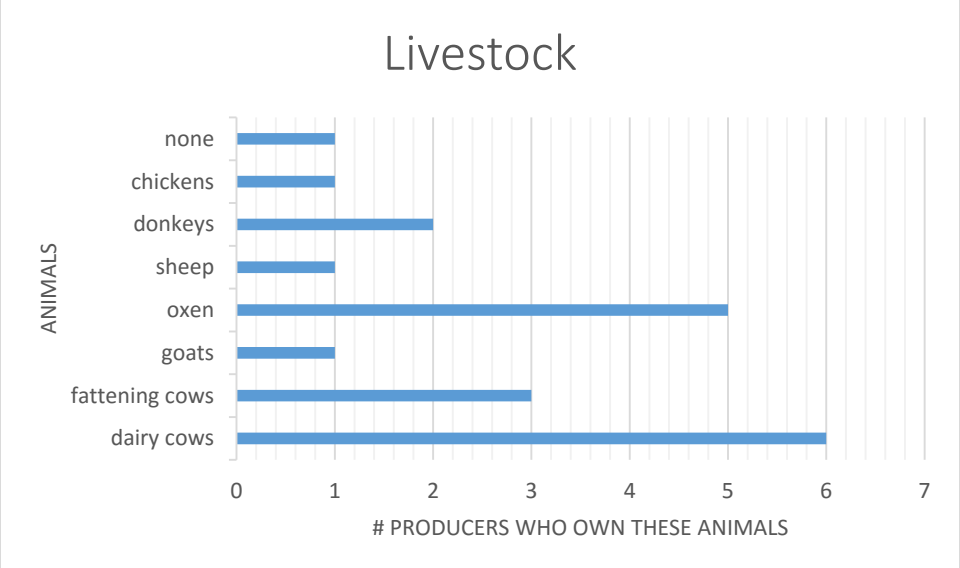


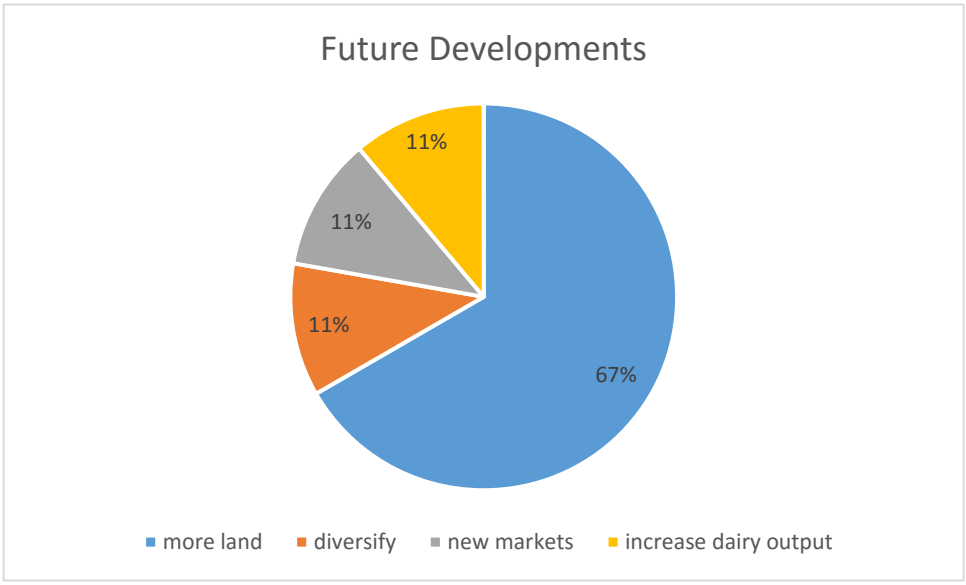
Producer	**Best Seller** and other forage
1	**pennesitum parum,** guatemala grass, napier grass
2	**alfalfa**, **vetch,** cowpea, rhodes grass, pennesitum parum, sesbania
3	**alfalfa**, lablab, desmodium, napier grass, vegana
4	**pennesitum parum**, napier, bana, other grasses
5	**rhodes grass**
6	**pennesitum parum, rhodes grass, alfalfa, guatemala grass,** napier grass, desmodium, vetch, cowpea, lablab,
7	**panicum,** pennesitum parum, cowpea, pigeon pea, lablab
8	lablab, cowpea, rhodes grass, bracharia, panicum, napier grass (1st year)
	panicum, rhodes grass, alfalfa, cowpea, lablab, napier grass (1st year)

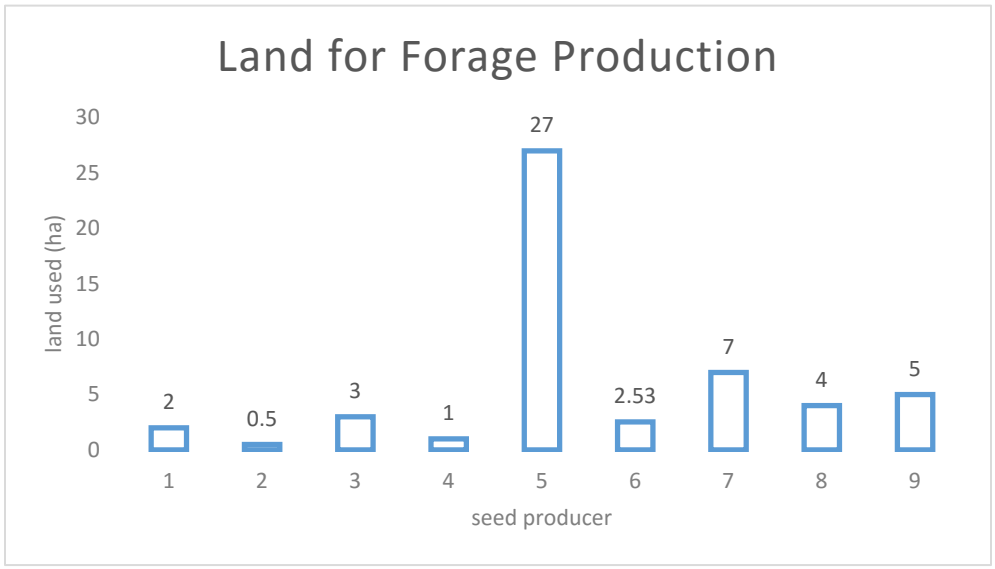


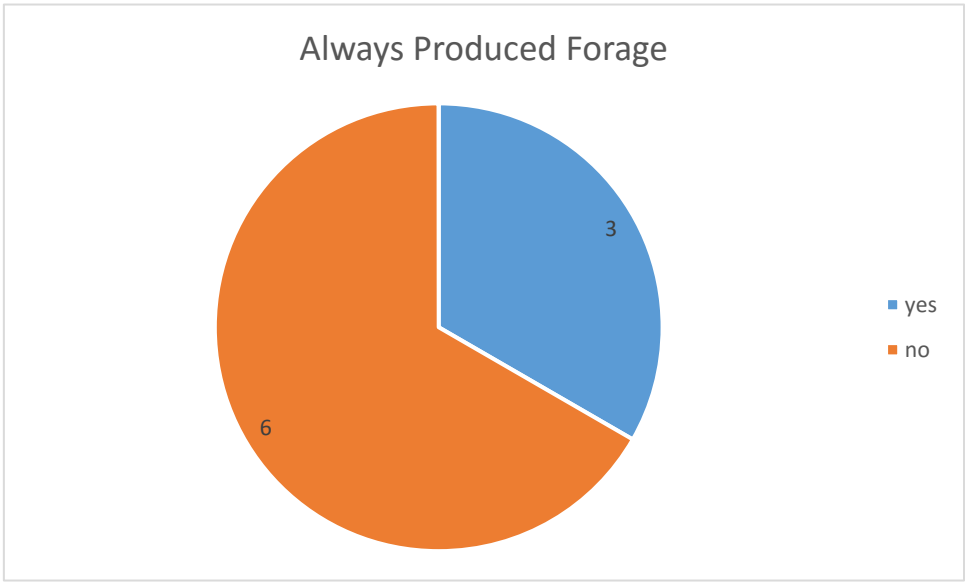

GENDER OF SEED PRODUCER
COUNT
2
7
FEMALE
MALE
GENDER


Supervisor:Laborer Ratio
100%
80%
60%
40%
20%
0%
ratio
1
2
3
4
5
6
7
8
9
seed producer
supervisors
laborers


Livestock
none
chickens
donkeys
sheep
oxen
goats
fattening cows
dairy cows
ANIMALS
0
1
2
3
4
5
6
7
PRODUCERS WHO OWN THESE ANIMALS


Future Developments
11%
11%
11%
67%
more land
diversify
new markets
increase dairy output


Land for Forage Production
land used (ha)
30
25
20
15
10
5
0
2
0.5
3
1
27
2.53
7
4
5
1
2
3
4
5
6
7
8
9
seed producer


Always Produced Forage
3
6
yes
no

Pictures

1) Forage Diversity- ILRI
2) Planting in Debre Zeyit
3) Looking through the microscope
4) Dissecting *Sesbania* flowers
5) Boiling brilliant blue stain
6) Planting in Addis Ababa
7) Women's co-op in S Ethiopia- with Asebe & Kayla
8) ILRI Addis welcome sign
9) Farm near Hawassa
10) Getting coffee in Butajari
11) ILRI Soddo- with Kayla
12) Adding fixative to *indigofera wightii* pistils- with Andreas
13) ILRI Zwai
14) Interviewing at a farm near Soddo
15) Hand-pollinating *indigofera wightii*- with Dr. Jean Hanson

(From left-right, top-bottom)