

The Field Guide

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1) Background information

The overall aim of the world herbivory project is to collect standard information at a wide range of sites, in all sorts of ecosystems around the world, in order to answer some big questions in ecology.

The first question we will address is: **“Is there a latitudinal gradient in the proportion of a plant’s leaf area that is removed by herbivores?”** (in other words, do animals eat more of the leaves in the tropics?). Most ecologists think that there will be a latitudinal gradient in herbivory, and in the strength of other plant-animal interactions. This theory is based on the idea that the generally favourable physical environment in the tropics, combined with the long ecological history of tropical ecosystems (which don’t get covered by glaciers every 10,000 years like more polar ecosystems) mean that plant and animal populations can build up to the point where interactions with other species limit their growth. In ecosystems closer to the poles the more challenging physical and environment and disrupted ecological history mean that populations do not build up to such levels. So, interactions between plants and animals are expected to be more intense in the tropics. The stronger interactions are thought to contribute to the greater biodiversity of tropical ecosystems. Of course, there are counter-arguments to the idea that plant-animal interactions will be more intense in the tropics. My favourite is the idea that the greater productivity of plants in tropical environments might be matched by a greater abundance of herbivores. If this were the case, there would be no latitudinal gradient in herbivory. One of the nice things about this project is that it doesn’t matter what result we find – the results will be interesting, and will have implications for other areas of ecology regardless of the outcome.

The second main area we will look at is the causes of global patterns in herbivory. **We will look at the relationships among:**

- **environmental conditions** (which determine the amount of resources that plants have to allocate to growth and defense),
- **plant traits** (especially those that influence the palatability of leaves),
- **herbivore abundance**, and
- **herbivory**.

We will also measure global patterns in seed predation (another form of herbivory), to see if we get similar patterns.

With this information, we will provide broad-scale tests of the many hypotheses about the factors that determine the amount of herbivory plants sustain. By increasing our understanding of the factors that influence plant-animal interactions now, we will vastly improve our ability to predict what might happen to these interactions under global climate change.

2) Herbivory

The most important thing we are measuring in this project is the proportion of a plant's leaf area that gets eaten by herbivores in a given amount of time.

How often should I measure herbivory? You should measure leaf loss at 3 monthly intervals though one year. This will allow us to quantify the seasonality of leaf loss in your area (which is important, because I suspect that the degree of seasonality will be one of the important differences between leaf loss in the tropics, and leaf loss in the arctic). This sampling regime does not allow us to quantify between-year variation in herbivory. While we acknowledge that this would be good to do, we simply do not have the time or resources to follow each site through multiple years. However, we will try to place as many of the sites as possible in areas where there have been ongoing studies of herbivory.

Which species should I study? It is not possible for us to study ALL of the plant species at every site. So, we have decided to study just the 4 most abundant (with the most canopy cover) species at each site. We will make estimates of the cover of the most abundant species, and nominate the 4 study species during the first visit to the site. At this time, we will also tag, and map the location of at least 5 individuals of each species. You should use these mapped plants for the herbivory study.

What part of the plant should I study? You should measure "leaf" loss on the photosynthetic organs of the plants, regardless of their true botanical form. So, you should measure phyllodes, or cladodes (flattened petioles and stems) as if they were leaves. If your study species has compound leaves, you should measure leaf area loss on individual leaflets.

IF you are wearing insect repellent or sunscreen in the field, please wash your hands before you start tagging, so that you don't get insect repellent or sunscreen on the leaves!

Which leaves should I tag? Tag recently-produced, fully-developed leaves, growing in full sunlight, without obvious signs of pathogen attack or herbivore damage. IF you are working at a site where it is not possible to reach sun leaves, then you will have to tag leaves on understory plants. However, I would still like you to aim for leaves that are in relatively high-light parts of the understory if possible.



How many leaves should I tag? At each of the four sample times, you should tag 3 leaves on each of at least 20 individual branches/stems (usually 4 branches on each of 5 individual plants) of each of the 4 study species (giving a total of 60 leaves per species per sample time).

How do I tag the leaves? The study plants will be marked with plastic flagging tape (and aluminium tags in fire-prone sites). The study branches should be tagged using white twist-ties. You can use the marker pens to put different numbers of stripes on these twist ties, to label the tagged branches. The study leaves should NOT be disturbed or drawn on. Instead, put a mark on the stem, just below the

petiole of the study leaf. If you are working with a woody species, you will be able to use the pink paint-marker for this (these leave the most visible permanent marks). However, if the leaves are on a non-woody stem, you should use the xylene-free markers to indicate the study leaves (these are less damaging to soft tissue). IF your study species has many, closely-packed leaves (so that it is not possible to get a marker pen to the stem), you should simply mark the leaf below the study leaf (taking care not to get ink on the surface of the focal leaf). For leaves with distinct upper and lower surfaces, you should monitor damage on the upper (photosynthetic) surface. For bifacial leaves (ones that hang vertically and photosynthesise with both sides), put a small dot on the surface for monitoring. The three leaves on each branch do not need to be numbered – leaf 1 is the closest to the growing tip, leaf 2 is in the middle, and leaf 3 is closest to the main stem/base of the plant.

Which side of the leaf should I study??

We will normally follow the fate of the upper surface of the leaf. If leaf looks the same on both sides (it is bifacial), you should leave a small dot on the sample surface.

	
<p>Tag each branch with a single, marked twist-tie. Make sure that the twist tie is far enough away from the tagged leaves that it won't damage them in the wind.</p>	<p>Indicate sample leaves with a blob of colour from a marker pen on the branch near the leaf.</p>

Potential complications in tagging, and how to deal with them:

My plant's leaves have indeterminate growth (e.g. grass leaves and cladodes). In this case, you will need to keep track of herbivory on a known region of the "leaf", rather than on the whole lamina. You should mark the boundaries of this region with the xylene-free marker pens.

My plant doesn't HAVE any leaves in this season! Some study species (especially deciduous or ephemeral plants) will not have leaves present at all of the sample periods. Obviously, it will not be necessary to sample these species at times when they do not have leaves. You can go home early!

All of the leaves on this species are about to senesce (fall off)! If all the leaves are old, and nearing the end of their likely lifespan, then don't bother tagging the leaves this season. This rule is designed to avoid confusing traumatic leaf loss (from things like herbivory and trampling) with programmed leaf loss (natural ageing and senescence). This distinction is important, because in the first case, the plant loses all of the nutrients previously invested in the leaf, while in the second case, the plant gets to resorb a significant proportion of the valuable nutrients.





Some leaves were damaged before I started! You should aim to tag relatively healthy, recently-developed leaves wherever possible. However, sometimes you will not be able to avoid tagging older, more damaged leaves. In these cases, you need to take a "before" picture (using the technique described below) to allow us to measure the extent of the initial damage.

I can't get through tagging this many leaves in one day! If this is the case, estimate how many you can do, and just do those. Please do at least 3 leaves on 4 branches on 3 individuals of each species.

How long should I leave the leaves before I measure how much area was lost?

In each season, you should aim to go back to the study site exactly 14 days after the leaves were tagged. Of course, this will sometimes not be possible (for instance in unworkable weather conditions). In these cases, you will have to use your own judgment about the best time to go out. In general, anything from 12 to 16 days will be o.k. – just make sure you clearly indicate how long your sample interval was. IF conditions are bad for too long (if the sampling interval exceeds 18 days), you will need to re-tag the leaves and try again when conditions are better.

What should I measure on the 14th day? Revisit all of the tagged leaves. Write down whether each leaf is undamaged, or if it is completely missing. If the leaves have been partially damaged, you will need to take a digital photo of the leaf, so that we can assess what proportion of the leaf has been lost. In these cases, take an image of the plant number, an image of the branch number, then a picture of an appropriate number of your fingers to show which leaf number this is, before you take a picture of the leaf (So I can figure out which leaf is which from the picture files). Don't worry about sorting out the file names – I can do that.

			
1) Take a picture of the plant tag, showing species and plant number	2) Take a picture of the branch tag, to show which number this is	3) Use fingers to indicate leaf number.	4) Then take a picture of the leaf.

Put the images in folders clearly labeled with the month, and whether the images were taken at the start or the end of the measurement period (e.g. folder/site_name/October/start, and folder/site_name/October/day14)

I will put up a website to receive images as soon as possible. I'll send you details as soon as I've got it working. If there is a delay of more than a couple of days before you send me the leaf images (for instance if you wait until the end of the study to send me the pics from all the seasons), please burn them to a cd, so we have an emergency back-up copy.

What sort of damage counts as "loss"? You should record all damage that would stop the plant from photosynthesizing with that part of the leaf, including lamina loss, leaf mining, and pathogen attack. If unsure, take an image.

How do I take the photos? First, take the plant, branch and leaf identifying images (See above). Then take the picture of the leaf. Set your camera to its macro setting (normally represented by a flower). If you are working in a shady understory, you might need to turn the flash on – but make sure that any images of glossy leaves are o.k. with the flash. Please take the pictures against the gridded background provided so that I can work out the actual leaf area remaining. Hold the leaf and grid as flat as possible (perpendicular to the camera) while you take the picture, and make sure the leaf is pressed against the background. Make sure that the resulting image has at least one visible square (for scale).

Potential complications in assessing herbivory, and how to deal with them:

The leaf was taken from the plant, but not eaten. You should still record the leaf as lost. This study attempts to assess the cost to the plant (in terms of leaf area lost), rather than the benefit to the herbivore (leaf area ingested). So, all leaf loss should be recorded, even if animals have damaged the plant without actually eating the leaves. It is possible that some leaves will be naturally senesced during the sample period. These leaves will be falsely recorded as "lost". However, the fact that we are tagging only the most recently-matured leaves, and revisiting the leaves 2 weeks after the initial tagging in each season should minimise the error due to natural senescence.

I'm sure there will be plenty more complications I haven't thought of yet. I will be checking my e-mail (amoles@bio.mq.edu.au) regularly – so just write to me and ask if you have any questions.

What data should I send you?

Send me your field notebook (by post), and your leaf images (either electronically, or by posting cds). If possible, please make a photocopy of your field notebook and spare copies of any cds before putting them in the post (just in case!). You will find instructions for sending images to me at the end of this document.

Herbivory during leaf development

A huge proportion of lifetime herbivory happens while leaves are still expanding. In this part of the project, we will estimate leaf area loss during development by taking images of leaves that have just completed development.

You probably only need to do this sampling in one of your four seasons – the season when leaves have most recently finished growing (spring, at most sites). However, if your species produce leaves continuously, you will need to take pictures in all seasons. To decide whether a species has suitable leaves, look for leaf sequences that have some leaves that are still growing.

What do I take a picture of?

First, take a picture of a plant label for the species (so I don't get confused!). Look down the sequence to the first leaf that has finished development (as well as being full-sized, this leaf will usually be a darker green than the "immature" leaves, and might be a bit tougher). Look very carefully at the stem, to see if there is any evidence for leaves missing from the sequence. If the first fully-mature leaf is partially damaged, take a picture of it. If the leaf between the last expanding leaf and the first fully-mature leaf is missing, take a picture of your fingers in an O shape, or put a note in your notebook. If the first fully-mature leaf is entirely intact, take a picture of one finger, or put a note in your notebook. Take pictures of at least 20 leaves, from a minimum of 5 individual plants.

3) Pre-dispersal seed predation

Which species should I include? Each season, check the 5 study species at each site to see if any of them are fruiting. If so, and if there are more than 50 seeds, spread across a minimum of 5 plants, then you should assess pre-dispersal seed predation on that species. To do this, collect 50 recently-matured seeds, from at least 5 plants. (I ask for recently-matured seeds because I suspect that predated seeds spend longer on the parent plant than healthy seeds. IF this is true (I don't have data on this), and IF you collect seeds that have been hanging around on the plant for a long time, you will over-estimate pre-dispersal seed predation). In species with multi-seeded fruits, you should collect

seed from a minimum of 20 fruits (from at least 5 individuals). If there are no suitable species in a season, you don't need to do anything. Otherwise, keep reading!

What part of the fruit should I study? The units of study here are true seeds (endosperm + embryo + seed coat), not the whole diaspore (ie, the apple seed, not the whole apple). The only exception to this is species whose fruit-tissues are firmly-fused to the seed coat (e.g. the achenes of Asteraceae). If you are unsure which fruit part is which (this would be totally understandable!), please ask Angela, or your local advisor.

How do I know whether a seed has been predated? It will often be obvious that seeds have been predated. In general, if the seeds have an obvious exit-hole, or if they have been replaced in the fruit by a pile of sawdust-like frass (insect poo), or if they have obviously-fatal chunks out of them, you should simply record them as predated. If they appear sound, you should take them back to the lab, and chop them in half with a razor blade (be very careful for your fingers...). You may need a hand-lens or microscope for some species. If you still see no evidence of seed predators after chopping the seeds in half, count them as sound. The tricky cases are those where the seeds have been a little bit nibbled at the edges. In these cases, you will have to use your own judgement. If the proportion of the seed reserves that has been removed is very small, and the damage has left the embryo intact (ie, if it looks like the seed has a very good chance of successfully germinating), then you should record the seed as sound. Otherwise, record the seed as partly predated.

What do I record if there were multiple seeds in a fruit, and they've all been destroyed? If all of the seeds in a multi-seeded fruit have been completely destroyed by a seed predator (i.e. are not able to be counted), then you should record the number of seeds preyed upon in that fruit as the mean number of seeds set per fruit for that species.

What output should I send in? Send in a tally of predated, partly-predated and sound seeds, for each of the 4 study species that set fruit in each season.

Pre-dispersal seed predation by taxa that remove the entire fruit from the plant will not be assessed.

4) Post-dispersal seed removal

Which species should I include? In each season, check whether the 4 study species at each site are setting seed. If so, and if there are at least 50 recently-matured, apparently sound diaspores available, and if the diaspores are suitable (see below) you should assess post-dispersal seed predation. If there are no suitable species in a season, you WILL still need to assess seed removal on the "standard" seeds, so skip to the section on "standard seed" below, and keep reading!

What part of the fruit should I be studying? We are trying to mimic natural exposure to post-dispersal seed predators. Thus, the unit of study is the part left exposed to post-dispersal seed predators after primary dispersal. In fleshy-fruited species (usually dispersed by vertebrates), you should remove the fruit pulp, and leave whatever is inside the woody unit (this may be one or more seeds) exposed to predators in the post-dispersal seed predation trial. Seeds that remain inside an intact fruit after primary dispersal should be put out inside than fruit, even if that means you are putting out a several-seeded fruit. Seeds that are normally released from the fruit before dispersal (e.g. most beans) should be removed from the fruit before exposure to post-dispersal seed predators.

Suitable diaspores: We have decided to place the seeds directly on the soil surface, in order to mimic the natural conditions as closely as possible. However, you will quickly discover that this

imposes a minimum practical size on the diaspores that you can use for post-dispersal seed predation trials (this size will depend on the texture of your soil, and will be influenced by the amount of colour contrast between the soil and the seeds). In short, if you have trouble seeing the seeds when you've just put them on the soil surface, you shouldn't try to assess predation on them! The other thing that can rule a species out from the post-dispersal seed predation trials is the effectiveness of their wind-dispersal adaptations, under the conditions at your site. In short, if you can't be sure whether a diaspore has been blown away, or carried away by a seed predator, you shouldn't try to assess post-dispersal seed predation on it (this criterion means we may get an under-representation of species from the Asteraceae in this part of the study, but we'll live with that!).

When should I collect the seed? Preferably on the day you do the trial (while you're at the site). Do not store the seeds for more than two days before using them in trials. IF you find seeds of a species that were not fruiting when I visited, please collect an extra 20 seeds/fruits and send them to me.

Standard seed: We are also gathering data on the proportion of "standard" seed removed by post-dispersal seed predators in each season at each site. This 1) lets us see how much differences in seeds between sites are influencing our results, and 2) lets us assess seasonality in seed predation, even if none of the study species are actually setting seeds in a given season. The standard seeds are pearl barley. We chose this species because 1) pearl barley is relatively easy to find in different countries, 2) it does not have particularly nasty physical defenses, and 3) because it comes from a very widespread family (Poaceae) – thus, they are less likely to have chemical defenses that your local herbivores have never encountered than are species from less widely-distributed families.

Depot location: Where possible, establish the depots in naturally-formed depressions in the ground (because disturbing the soil has been found to increase invertebrate activity in an area). If there are no suitable depressions available, try to create a depression with the least possible disturbance to the soil surface. The depressions should be just deep enough to reduce the chances of the seeds blowing away. If there is a continuous bed of leaf litter or plant material, you will need to clear a little patch before setting out the seeds. Try to keep the disturbance as small as possible.

Arrange the depots at about 5m intervals along one or more straight transects through the study site (ie, walk to a spot 10m down the transect line, then look for the nearest suitable depression). Make sure that these transects remain within the same type of vegetation as the seeds came from (if necessary, make several parallel transects across the study site).

LAY THE TRANSECTS THROUGH THE SAME SORT OF VEGETATION AS YOU HAVE TAGGED PLANTS IN. IF POSSIBLE, LAY THEM SLIGHTLY AWAY FROM THE HERBIVORY PLANTS, SO YOU DON'T STAND ON THE DEPOTS WHILE MEASURING LEAVES. IF THIS IS NOT POSSIBLE, SET THE DEPOTS UP IN THE SAME AREA AS THE TAGGED PLANTS, BUT SET THEM UP AFTER YOU FINISH TAGGING, AND COLLECT THEM IN AT THE START OF THE DAY 14 DAYS LATER.

Species arrangement: arrange the diaspores from different species randomly along the transect line. I usually achieve this by labelling all the flagging tape first, mixing it thoroughly in a bag, then randomly drawing one piece at each depot.

The depots: Establish 10 depots for each species on the day you tag leaves for herbivory. At each depot, put a toothpick in the centre of the depression, then put 5 diaspores of ONE SPECIES within 5cm of the toothpick (if your diaspores are too big to fit in this area, you can put them further away – but make sure they are as close in as possible). Set the seeds directly on the soil surface (to mimic the

natural situation as closely as possible). Put a piece of labelled flagging tape up about 1m away (no closer, in case it scares or attracts seed predators; no further away, in case you can't find the seeds again!).



The perfect seed depot. The seeds are all close to the toothpick, and the soil surface is undisturbed.

When should I assess removal? If you are easily able to visit the site the day after the seeds have been set out (e.g. if the site is close to a second site you are measuring), it would be great if you could walk the transects and count the number of diaspores remaining at each depot 24 hours (plus or minus 2 hours) after the seeds were set out. Do this **WITHOUT** disturbing the depots, or replacing missing diaspores. This 24 hour assessment would be good to have, but is not absolutely essential. However, it **IS** essential that you assess the number of diaspores remaining at each depot 2 weeks after the diaspores were set out.

How do I assess removal? Walk along your transect, and count the number of diaspores remaining within 30 cm of the toothpick at each depot. Record missing diaspores as “removed”. If any of the remaining diaspores have been damaged to such an extent that germination seems unlikely (ie if a large proportion of the reserves have been removed, or if the embryo has been seriously damaged), you should record the seed as “damaged”. Record the remainder of the diaspores (sound diaspores that are still there), as “remaining”.

NOTE: This method quantifies post-dispersal seed removal, but not post-dispersal seed predation *per se*. This is definitely not perfect. However, techniques for following seed **fate** (such as radio tracking and thread following), are simply too labour intensive for use in a study of this size.

5) Invertebrate abundance

One of the things we need to find out is how many herbivores there are at each site. We can't gather new data on vertebrates (too hard, but we'll use whatever previous data are available for each site), but we can quantify invertebrates, using standard pyrethrum spraying.

How many samples shall I take? Sample three branches of each of the 4 most abundant species, at four times throughout the year (once in each season). Sampling should be done in the morning.

How much foliage do I spray? To standardise across all the different growth forms, we have decided to sample a standard surface area of foliage: 20 cm by 20cm (400cm²). To figure out how many leaves of each species to spray, divide 400 by the average leaf area (in cm²) of that species. Then, count out that many leaves from the growing tip of a sun twig (include side branches; and feel free to use estimates based on the number of leaves per 5cm of stem if your species has very small leaves), and put a mark on the branch. For grasses, sample an appropriate area of leaf lamina (don't try to sample right down to the base of the leaf). For species with flattened stems, work out the average width of the stem, then calculate the total length of stem needed to make up the sample.

How do I sample invertebrates??? Mix 30ml of the pyrethrum concentrate with 5 litres of water, and put it in a sprayer. Place polythene sheets under the sample branches, trying not to shake the plant as you do so (because this would make the flying insects take off). Pump up the pressure in the sprayer, and mist the foliage until it is wet all over. Leave foliage to drip for a few minutes, then return and shake the foliage over the polythene sheet (to dislodge any invertebrates sticking to the plant). Label one 70ml sample jar for each branch (include site name, date and plant species on the label). Now, use a paintbrush to sweep the invertebrates into the sample jars. Add 100% ethanol so that the sample is stored in at least 70% concentration ethanol.

What do I do with the samples? At the end of the study, pack the jars securely, and give them to your advisor (my collaborator in your area) to post to me. At a later date, samples will be sorted to broad feeding guilds (herbivores/predators/omnivores), and the number and mass of individuals will be quantified. Store sample jars upright whenever possible, to minimize leakage.

6) Selection of study individuals

Most of the study individuals will be tagged at the beginning of the study. However, you will need to replace any individuals that die during the course of the year (unless this is a normal part of that species' life cycle). You can choose any new individual to tag, as long as it is more than two canopy-diameters away from the nearest plant of the same species, and looks reasonably healthy, and outwardly "normal" for its species.

7) At the end of the study

Please remove all the twist-ties, flagging tape, plant tags, wire, etc from the study site on your last visit. There is no chance we will repeat this study, and I don't want to be responsible for leaving non-biodegradable marks at study sites all around the world!

8) The Quick Guide.

On the first field day of the season you should:

- Tag the leaves for herbivory (3 leaves on each of 4 branches on each of 5 plants of each species).
- Collect any available seeds, and assess pre-dispersal seed predation
- Put out the post-dispersal seed removal trials (at least for standard seeds)
- Take pictures of recently expanded leaves (if there are any around)

On the follow-up field days you should:

- Count the proportion of seeds remaining in the seed predation trial.
- Assess herbivory on the tagged leaves (note if each leaf is undamaged or completely removed, take a digital picture if it's partially damaged)
- Spray for invertebrates.

9) Any questions?

IF YOU ARE UNSURE ABOUT ANYTHING, PLEASE ASK!!!

If it's an easy question, you should talk over the options with your local collaborator. However, remember that it is **CRUCIAL** that the sampling is done in a consistent way across the sites. I would much rather have you ask me for advice in tricky situations than decide on your own. I will be checking my e-mail as regularly as possible while I travel the world (I should always manage a weekly check), and I will do my best to get back to you quickly.

e-mail me at: amoles@bio.mq.edu.au

How to send in your images

1) If you need an FTP program, start by downloading AceFTP free from:

<http://software.visicommedia.com/en/products/aceftpfreeware/>

(Mac users: I'm afraid I haven't looked for a freeware program for you – let me know if you have any suggestions).

- The rest of the instructions are written for people who are using AceFTP – but they should translate to your favourite program without too many hassles. I've put the really important bits in bold.

2) Set up a new connection profile. In AceFTP, do this by clicking on the FILE menu option, then CONNECT, and click on NEW SITE PROFILE.

Call the new site something sensible like “Herbivory image repository”

The **server** you need to connect to is called: ITSCS02.OCS.MQ.EDU.AU (*that's a zero before the 2, not an oh.*)

The **username** is: .worldherbivory.users.els.mq (*don't forget the dot at the start!*)

The **password** is: herbivore

In Ace FTP, click the box to connect to the site when you finish, and then click on the NEXT tab.

3) AceFTP will now ask which folders you want to use.

The **local folder** is whichever folder on your computer you have put the herbivory images in. You can browse for this in AceFTP by clicking on the little folder icon to the right of the local folder box.

The **remote folder** is: /data2/els/bio/worldherbivory

4) Now connect! In AceFTP, you will be able to access this profile in future by simply clicking on FILE, CONNECT, then clicking on the arrow to the right will bring up a list of remote sites, including the Herbivory image repository (or whatever you called that profile in step 2!).

5) To transfer the images, browse your local folders (shown in the window on the left in AceFTP), and select the relevant folder.

NOTE: Please label the folders with the appropriate site name and region. Remember to include the month the pictures were taken in the folder name, and indicate which pictures are initial images, and which are from the 14th day.

Now, click on the arrow in the centre of the screen to transfer the whole folder across to the data repository. Please include the folder – I'll get very confused if everyone sends 500 images that aren't packaged in folders!

It might take a while for the images to be transferred – don't panic!

6) You should be able to see the files in the remote folder. However, you will not be able to change them, or delete them.

That's all! You can disconnect now.

THANKS!