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06/06/2006 11:52 AM
AST

To: <asreeve@maine.edu>, <mrasher@ftw.nrcs.usda.gov>, <rachel.novick@yale.edu>, <hilary_neckles@usgs.gov>, <ring.carde@ucr.edu>, <Martin.Vandergrinten@ny.usda.gov>, cc: Subject: Re: National Park Service Investigator's Annual Report Past Due for Your 2005 Research @ Acadia National Park

Thank you for the reminder; I'll try to get you something. Can I send you for now this NSF project proposal which basically summarizes what we have done so far? I have attached it here.

Jonathan Adams

----- Original Message -----

From: <David_Manski@nps.gov>

To: <jonadams@andromeda.rutgers.edu>; <asreeve@maine.edu>; <mrasher@ftw.nrcs.usda.gov>; <rachel.novick@yale.edu>; <hilary_neckles@usgs.gov>; <ring.carde@ucr.edu>; <Martin.Vandergrinten@ny.usda.gov>

Sent: Tuesday, June 06, 2006 10:39 AM

Subject: National Park Service Investigator's Annual Report Past Due for Your 2005 Research @ Acadia National Park

> During the end of December 2005, you were sent an automatic email message
> about the need to submit your National Park Service Investigator's Annual
> Report covering your 2005 science activities at Acadia National Park.
> Although due by March 31, to date, we have not received your report. As a
> condition of your research and/or collecting permit we asked that you
> complete this report. This email is a reminder that we still are very
> interested in a summary of your project work last year (even if that work
> involved data analysis/report writing). Park staff and the public are
> interested in the status of many of the park's research activities and
> your
> annual report is one of the primary ways I can keep everyone updated on
> your work. Instructions on submitting your report are detailed below.

> Thank you for your cooperation.

> David Manski
> Chief of Resource Management
> P.O. Box 177
> Bar Harbor, ME 04609
> 207/288-8720 (voice)
> 207/288-8709 (fax)
> david_manski@nps.gov

> (See attached file: IAR_call_investig05_tg_jd_fnl.doc)



NewLatLeafLoss_MAINTXT5thMay2006.doc

Latitudinal gradients in leaf herbivory damage in four temperate tree species

Introduction

It is widely believed that intensity of herbivory is greater at lower latitudes (e.g., Dobzansky 1950, MacArthur 1969, Coley & Aide 1991, Coley and Barone 1996, Salgado & Pennings 2005, Pennings et al 1996). Although not often stated explicitly, the expectation is that in more seasonal climates with hard frosts, winter 'knock back' of insect and other herbivore populations should be more severe, such that herbivore populations less often reach densities limited by food supply or parasitoids/diseases during the growing season. The gap between potential and actual population levels is believed to translate into less herbivory in higher latitude, more seasonal locations.

If there is indeed a general latitudinal gradient in herbivory, this has implications for many aspects of community and ecosystem functioning, including species diversity, nutrient cycling and co-evolution (Janzen 1970, MacArthur 1969, Coley and Barone 1996), and the future behaviour of communities in response to climate change (IPCC 2001). It has been suggested that herbivory gradients in relation to temperature are also detectable in fossil leaf deposits (Wilf and Landabiera 1999). Because of greater herbivore pressure, plants are in turn thought to be more highly defended, or otherwise less palatable, at low latitudes as a result of natural selection (e.g., MacArthur 1969, Hay and Fenical 1988, Coley and Aide 1991, Coley and Barone 1996, Salgado & Pennings 2005, Pennings et al 1996).

Despite the general acceptance of such patterns, evidence for latitudinal gradients in plant defenses or plant palatability is fairly tentative, sparse and often contentious. Firstly, conclusions are often based on literature reviews rather than systematic studies using the same sampling methods (Coley and Barone 1996, Pennings et al. 2001, Andrews and Hughes 2005). Studies have compared different plant species/communities, rather than the same species over a gradient, making it more difficult to know what is directly comparable in terms of amount of herbivory. Thirdly, studies have often focused on damage by very different/local types of herbivores that are difficult to compare between regions (as noted and discussed by Pennings et al 2001, Coley and Barone 1996 and Andrews and Hughes 2005).

Indeed, if one examines the results of the very few *standardized* studies on geographical variation in herbivory in individual plant species, there appears to be little evidence for the expected trend with latitude. Swihart et al. (1994) found that snowshoe hares ate fewer aspen and birch twigs from boreal tree populations than those of more temperate populations, and suggested that a latitudinal trend might actually be present due to heightened plant defenses at high latitudes. However, a recent published study on a woody plant in eastern Australia compared insect herbivory latitudinally using standardized methods, and found no latitudinal trend in herbivore damage (Andrew & Hughes 2005).

Also, while many ecologists use altitude as a surrogate for latitude (e.g. Hodkinson 2005), altitudinal trends of herbivory are also equivocal. Evidence exists for greater herbivory at a higher altitude, a surrogate for higher latitude (e.g. Hengxiao et al. 1999), as well as greater herbivory at a lower altitude (e.g. Suzuki 1998).

Studies to date that examine herbivory systematically over large distances have been few and very limited in scope. For example the study by Andrew and Hughes (2005) concentrated on only one species and then at only four close clusters of sites along its latitudinal range. This study proposes to focus on four tree species across latitudinal gradients of 15-25 degrees of latitude, to more closely standardize the herbivory comparison.

Hypothesis testing. Essentially this work is intended to provide a much-needed test to the following hypothesis: that due to trends in mean annual temperature and in the length and intensity of the winter cold season, there will be a significant latitudinal trend in herbivore leaf damage within each tree species from north to south, with more southerly localities experiencing more leaf damage. The research proposed here breaks new ground by studying several tree species at numerous sites over a wide latitudinal gradient, using closely standardized methods.

An additional sub-hypothesis might be that herbivory will be more temporally and spatially *variable* in northerly regions due to the vagaries of knock-back of parasitoid and predator populations in regions with strong winter frosts and a long dormant season, allowing 'escape' of herbivore populations at some times and places (Turchin 2003). Alternatively the low species richness of the high latitudes might introduce chaotic herbivore population dynamics not seen in more species rich communities of warm climates. There may thus be a high site-to-site and year-to-year standard deviation in herbivory for more northerly sites (Turchin 2003).

Overview of approach

Study region. This study will be set along a latitudinal gradient through the eastern deciduous and mixed forest region of North America. It will also extend to the tropical montane cloud forest on the Sierra Madre Oriental of Mexico, in a climate which has features in common with eastern North America, except having less temperature seasonality. The intention is to compare herbivory damage over a gradient from weak cold seasons - without frost in the case of the Mexican forests and almost without frost in southern Florida - to the long and intense seasons of frost that exist for several months in the north-eastern USA and Canada, with temperatures as low as -30°C.

Although subject to varying biogeographical definitions, the temperate-mixed deciduous forest region stretches from approximately 50°N in Canada to 26° N in Florida, offering about 24 degrees of latitude, and variation in mean annual temperature from about 24°C in the south (Fig 2c) to 3°C in the north (Walter 2002).

A similar vegetation, with many genera and even species in common with eastern North America, exists more locally in the mountains of eastern Mexico. Montane cloud forest in Mexico is limited to a narrow strip in the mid-elevation of the main mountain ranges between 1200 and 2000 m of altitude, in the Sierra Madre Oriental and the Trans-Mexican Volcanic Belt. Many of the dominant trees occur both in this cloud forest and in the eastern deciduous forests of the United States. Arborecent genera common to eastern United States and eastern Mexico are: *Acer negundo*, *Carpinus caroliniana*, *Cercis canadensis*, *Cornus*, *Fagus*, *Ilex*, *Liquidambar styraciflua*, *Magnolia*, *Ostrya virginiana*, *Platanus*, *Styrax* (Graham 1973). Total annual precipitation in this montane region of Central Veracruz varies between 1300 and 2200 mm, while mean annual temperature is between 12 and 18°C. Typically, there are three well-defined seasons, the relatively dry-cool season lasts from October-November to March, a relatively dry-warm season during April and May, and the wet-warm season from June to September-October. In the Xalapa region (1300-1400 m altitude), mean temperature is 18°C, mean minimum temperature is 10.4°C, and mean maximum temperature is 28.5°C.

The moist climate throughout the eastern North American region allows one to study the empirical effect of a latitudinal gradient in winter temperatures and in seasonality without any strong complicating trends in dry seasons or relative aridity. The mountains of southern Mexico have even milder winters without frost and very weak temperature seasonality. They do have a weak dry season, but year-round rainfall is sufficient to ensure a closed mesic deciduous forest as the natural vegetation. In North America, the study will sample along a rough transect of around 70 sites passing

from Florida through the piedmont and coastal plain areas of the eastern USA, across lower parts of the mountains and plains of northern New England to the lower country of Quebec in the north.

The proposed transect generally follows parallel to the coast from north-to-south to avoid the confounding factor of differing continentality for areas of the same mean annual temperature. A fairly straight north-to-south route also offers the most efficient use of traveling time in covering a range of latitudes. However, at some points (e.g. northern New England) the transect does move further inland to more effectively sample north-south along the existing road networks, to sample over a range of soil types, and to pass through areas that are rich in one or more of the species selected for this study.

Analysis of individual species along latitudinal gradients. If the hypothetical effect of winter 'knock back' on herbivores really is more significant in the colder and more seasonal climates of the north, individual species of trees should be subject to less herbivory on average towards the northern part of their range as their own particular herbivores spend less time close to density-regulated population levels. For this study selected four common trees with wide latitudinal ranges in eastern North America. These are *Liquidambar styraciflua* (28-41° N, and to 19°N in Mexico), *Quercus alba* (30-46° N), *Fagus grandifolia* (30-48° N, and to 22°N in Mexico) and *Acer rubrum* (26-50° N) (Fig 2.1 and 2.2). The choice of these trees also incorporates a fairly wide taxonomic scatter among the angiosperms (Fagales, Sapindales and Hamamelidales).

Figure 1.1 (Below) Natural range maps of the tree species in this study. From top left, a) *Quercus alba* b) *Acer rubrum* c) *Fagus grandifolia* d) *Liquidambar styraciflua*. *Fagus* and *Liquidambar* also occur in eastern Mexico (see 1.2 below).

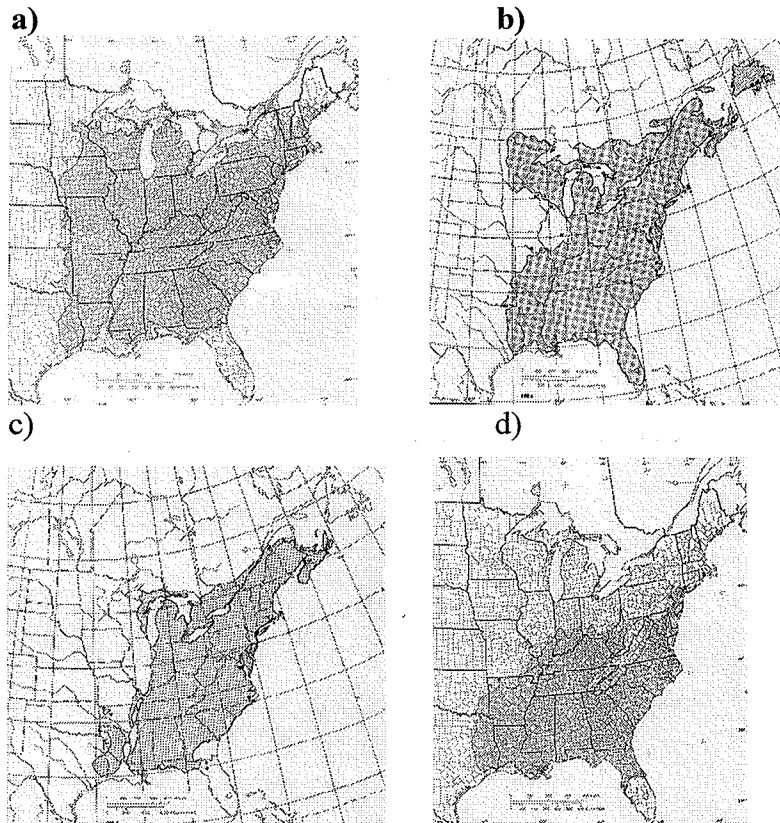
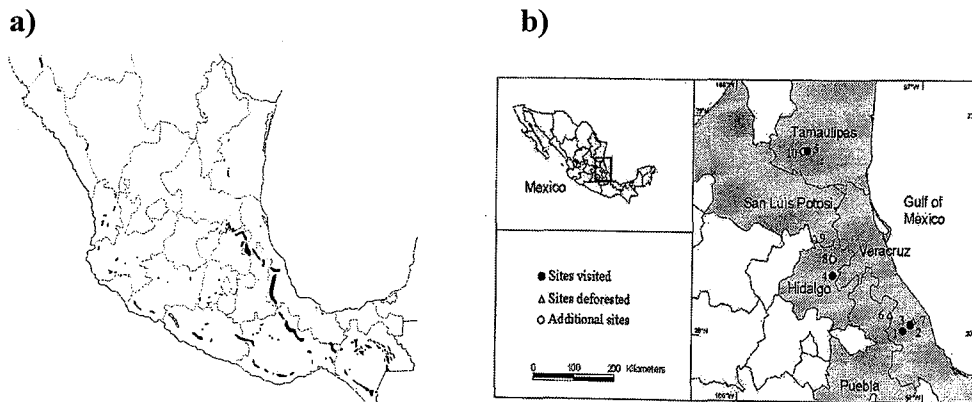


Figure 1:2 (below) Maps of Mexico with distribution of (a) *Liquidambar* and (b) *Fagus*. The sites of interest are scattered along the Sierra Madre Oriental, in eastern Mexico (boxed area in right hand map).



Sampling of sun leaves. There are many potential ways in which leaf samples could be selected. Varying studies of tree herbivory have concentrated on canopy tops using cranes, walkways or shooting samples down, on lower shade leaves of mature trees or shade leaves of understory young trees or seedlings (Coley and Barone 1996). In detecting geographical gradients, more important than the actual method of sampling is that the sampling is done consistently. The lack of consistency in available studies has been identified as a major problem in detecting latitudinal gradients in herbivory (Coley and Barone 1996, Pennings et al. 1996).

The proposed study will be based on assessment of insect damage of *sun leaves* from trees along forest edges. Edge sun leaves were chosen for study because it is very difficult to allow for differing precise degrees of shading and the movement of sunflecks over shaded leaves during the day. Leaves clearly out in the open in full sunlight for most of the day offer a more standardized sample.

While some work has suggested that forest edges and sun leaves suffer either more (Barone 1994, Hunter and Forkner 1999) or less herbivory (Sipura & Tahvanainen 2000, Lowman 1985), the fact that the sampling environment is quite closely standardized is the important issue, not whether the edge itself suffers more or less herbivory than the forest interior. The aim of this study is to test for the existence of *gradients* in herbivory, not to arrive at quantitative figures for rates of herbivory turnover in the whole forest canopy.

Sampling along forest edges as a standardized environment also offers the advantage that edges are more accessible than canopy tops or the shade leaves within the forest interior. This allows greater numbers of sites to be sampled per season. Leaves overhanging a small road can also readily be sampled in a few minutes without requiring permission from landowners, often a time-consuming process.

Duration of the study. The study is intended to last two years (based on two summer seasons of sampling), with the intention that allowing a combined data series over 2 years which can factor out some of the possible inter-annual variability (Turchin 2003), allowing a firmer conclusion.

Choice of sample locations. When comparing regions geographically, it is important to try to avoid bias in the spatial sampling towards particular environments or community types in different regions. However, even if one samples different regions in a spatially representative way some topographical and soil differences between regions are inevitable.

To generally avoid biases, we have chosen to scatter sites in a semi-random way along a 'grand transect', that includes lowland and piedmont sites in both its northern and southern parts. The

general outline of this transect can be seen in Fig.2a, although many more sites will be added to this total. Based on collecting strategies developed during the 2005 trial season, 10 day collecting trips will be planned along major north-south routes such as interstates, on a circular track, though areas known to contain abundant populations of as many as possible of the four selected species. Routes are also deliberately chosen to cross a range of soil/landscape types (e.g. both coastal plain and piedmont). State and national parks within 30 km of the major access interstate and state highway routes will be sampled (blanket permits have already been obtained for collecting), at points chosen randomly (a pin placed blindly on a map) within these parks. An equal number of additional randomized sites outside of parks are added by randomly placing a pin on each page of the state road atlas which the main interstate route passes through. The investigators will then drive as closely as possible to this random point and sample the nearest accessible forest edge sites containing one or more of the four species of interest, while bearing in mind the limitations of access to sites along road sides. Samples will all be taken along edges of small roads or tracks 5-20m in width (defined as the distance between the forest edges on each side), forested on both sides of the road, passing through forest areas >10 ha in total size. This method proved effective at locating sample populations during the 'trial run' in summer 2005.

In the larger contiguous areas of forest (e.g. the larger state and national parks), clusters of several (5-6) separate 'sites' may be located if more than 5 km from one another. Such clusters of sites will offer an interesting further analysis on the ways in which herbivory varies spatially of a hierarchy of scales.

Additionally, in the north-eastern USA some lower mountain sites (up to 400m) are included where the transect crosses large mountain belts (although even in northern New England most sites are in the flat or gently rolling lowlands rather than mountain ranges). In eastern Mexico, the sites are all at high altitude because *Liquidambar* and *Fagus* do not occur in the lowlands.

In Mexico, fieldworkers will sample scattered sites (at least 5 km apart) in both these species, according to accessibility. *Liquidambar* has high population sizes, and it is co-dominant both in the tropical montane cloud forest and old fields. *Fagus* has a restricted geographical distribution in Mexico, but it is abundant and dominant where it occurs. 15-20 sites of *Liquidambar* and at least 8 known sites of *Fagus* will be sampled in eastern Mexico.

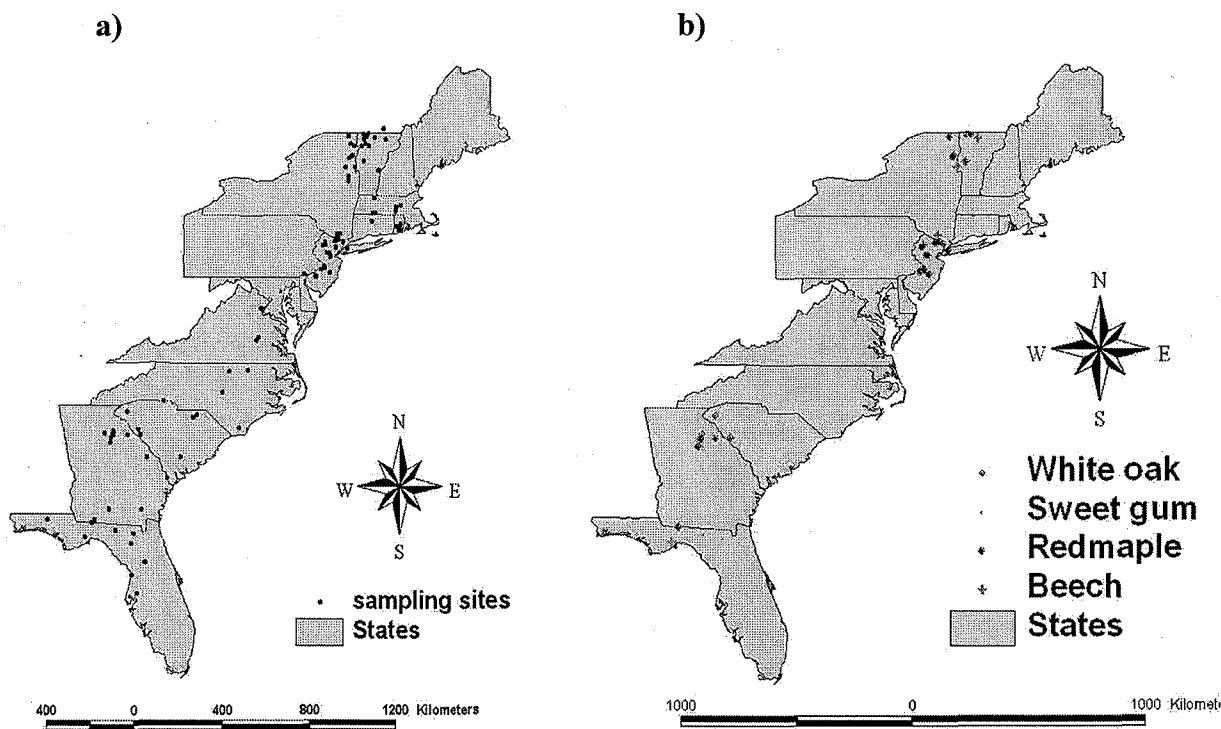
Although considerable soil and landscape heterogeneity is included in the sampling in both eastern North America and southern Mexico, the large number of samples taken will allow analyses of subsets of data (see section below, on analysis). The focus in this study on particular species (each having its own general niche requirements) will in a sense offer a self-selected sub-sample of soil characteristics that reduces heterogeneity among the sites, offering a clearer focus on climate as a variable along the transect. In the case of *Acer*, which grows in both swamp and upland habitats, we will endeavor to sample both types throughout the transect and note the sampling environment (swamp or upland) for separate sub-analysis.

We have found that with general field knowledge of the habitat preferences of each species, plus the aid of a GPS and some determined driving, up to 10 localities a day spaced more than 10km apart from one another can commonly be visited, and leaves of at least one species collected from each locality. Field collecting during 2004-2006 (on this project and separate projects by the PI relating to herbivory damage in leaf litter and tree seeds) has ensured a large database of sites to build from (Figure2 a, b), plus general experience in locating the types of areas in which each species will likely be found and be accessible. The working knowledge of areas of relative abundance of each species on both a broad and local spatial scale, plus observation of general landscape habitat preferences of each of the four species gained during our exploratory year, will greatly facilitate the

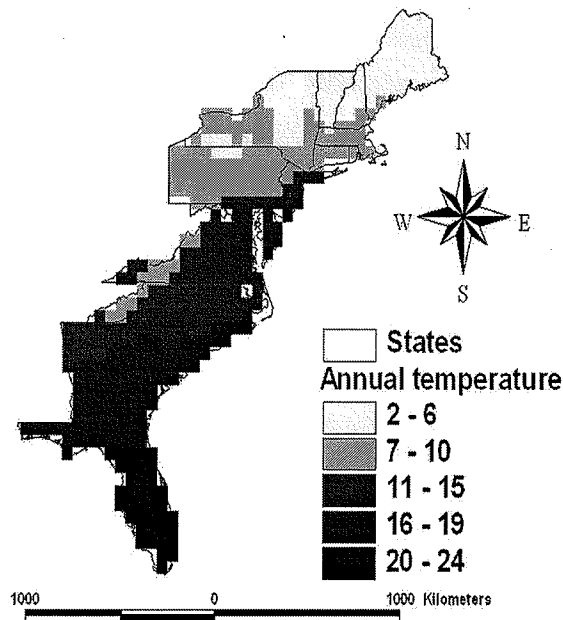
rapid location of further field sites.

On reaching a site, the first population encountered of each species satisfying the criteria (at least 10 individuals within a 100m forest edge along a road or trackway) is sampled. No further samples are taken from this 'site' (a 5 km radius of the last sample is defined as part of the location of this site; beyond a 5 km radius populations may be classified as a new and separate 'site'). Trees sampled are each marked with yellow plastic tape around the trunk to allow easy resampling the next year. A GPS reading is taken at each end of the transect and recorded.

Fig 2. (below) Maps of existing sites in the eastern USA from which the PI has already sampled populations of one of more of the four species of interest in this study, on projects on both live leaves and leaf litter. a) sites sampled in winter 2004/2005 and summer 2005. b) sites sampled for summer leaf herbivory only in the trial run of this project in 2005. In the proposed study all sites in 2a would be sampled in summer, and further sites added, including additional sites to the north and south of those shown here. The aim will be to ensure at least 50 sites per species, over the full latitudinal range of each species. Figure c) shows mean annual temperature in the eastern US region to be sampled. Sampling will also extend into Canada, and eastern Mexico.



c)

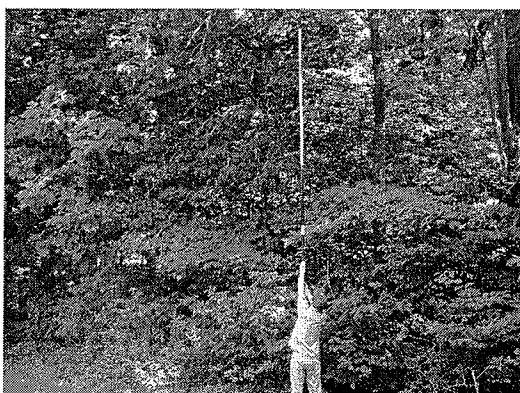


Details of methodology of sampling leaves along forest edges.

This approach, tested during the summer season of 2005, samples directly from trees, using a pole pruner at 4-5m height (see Fig.3). Sun leaf branches will be determined as those entirely illuminated in full sunlight when viewed during the middle hours of the day (10am-4pm). If a site is visited outside these hours, the position of the branch and likely east-west track of the sun above will be considered (using a compass or GPS) in order to determine whether a particular branch is likely to be exposed to this direct sun for at least several hours during the day (rather than a transient exposure, blocked by other branches above or the shade of the forest canopy).

Single species samples. For each potential 'site' located on a map, we will locate the nearest accessible forest edge population of each species to the center of the site. Depending on locality, because of the heterogeneous composition of the forest, this may be up to 5 km from the center of the site. Sampling will take place where 10 or more individuals of one of the 4 chosen species occur along a distance of at least 100m of forest edge. For example, sampling in the transect for *Quercus alba* will start by sampling a *Q. alba* individual at one end of the transect, skip 10m and then arbitrarily choose a branch from the next closest individual of *Q. alba*, then skip another 10m and sample again, and so on. For each individual sampled, a branch of around 30 leaves at about 4m height will be cut. This gives (for 10 individuals) about 300 leaves per site. Collectors will also be instructed to avoid making choices based on damage, and choose the first sunlit branch of the correct height within reach along the forest edge along the transect. Random sampling irrespective of herbivory damage is facilitated by the fact that each branch is too far up to accurately assess herbivory damage from the ground. Along the forest edge where each species is sampled, its relative abundance (as a % of the distance along the forest edge on each side of the road) in the forest community will be estimated. Other tree species and their relative abundances will also be recorded.

(Fig. 3. Below) Collection of sun leaf branches using a pole pruner.



Soil sampling. To help increase understanding of possible causes of geographical and site-to-site differences in herbivory we will take two 30cm soil core samples at each site, in the soil 2m from the trunk of the tree sampled, at either end of the transect. These will be analyzed using standard methods for texture, organic content, nitrogen and pH, by the technician employed plus undergraduate assistants. Soil depth to 50cm will also be estimated with a soil auger.

Timing of sampling during the growing season. Sampling at about the same time in all localities (north and south) would not only be logistically difficult but would also not take into account the major differences in phenology from north to south. The growing season in Florida or in eastern Mexico begins months earlier than in Quebec, and any tree (e.g. *Fagus*, *Acer rubrum*, *Quercus alba*) harvested in Quebec will have been exposed to several weeks less potential herbivory than one in Florida or Mexico. Thus sampling will take place in early summer (about 10 weeks after the mean bud break time averaged across a range of deciduous species) at phenologically analogous stages at different latitudes. Equivalent sampling times are estimated using two methods:

Method 1. (e.g. Li et al. 2002). Growing degree days (GDD) are calculated using the formula: $\{(T_{max} + T_{min})/2\} - B = \text{Growing Degree Days (GDD)}$ where B represents a base temperature value of 10°C. For the base 10/30 method, the following adjustments are made: 1) temperatures below 10°C are set at 10°C, and 2) temperatures above 30°C are set at 30°C. Climate station data are obtained from the following source: <http://www.engr.udayton.edu/weather/>

Method 2. Phenological delay is calculated using the predictive formula of Borchert et al. which has a predictive R² value of 0.97 for bud break time through North America and montane Mexico (Week of bud break = $10.3 \times (0.77 \times \text{mean temperature of coldest month})$). This empirical formula was based on observations all the way from montane eastern Mexico to Canada. While the formula characterizes bud break, we use the relationship between seasonal delay and temperature (using climate data from <http://www.engr.udayton.edu/weather/>) to determine equivalent delay in early summer sampling times 10 weeks after bud break, working northward from Mexico/Florida.

Table 1. (below) Starting from a 'standard' date of 10th July in New Jersey at 40°N, the following sampling times are estimated to be equivalent in terms of biological activity and leaf development, using two different methods. Where a range of dates is given, the earlier date is for the southernmost limit, the later date for the northernmost.

Latitudinal band	Latitude	Date from growing degree days (GDD)	Date from Borchert et al. formula
eastern Mexico montane sites	20-23°N	n.a.	7 th May

southern Florida, southern Georgia	26-31°N	12 th -28 th May	15 th -30 th May
northern Georgia, South & North Carolina	33-36°N	5 th – 17 th June	7 th – 15 th June
Virginia-Maryland-Delaware	37-39°N	19 th June-4 th July	24 th June-6 th July
New Jersey-southern New York State– Southern New England	40-42°N	10 th -20 th July	10 th -17 th July
Northern Vermont, Northern NY, Quebec	43-48°N	21 st -31 st July	20 th -30 th July

An average is taken between the estimated dates obtained by each method (which are generally within a week of one another), to determine sampling time at each latitude. The north-south transect in eastern North America will be divided into four latitudinal bands corresponding to approximately 10 weeks after the average time of bud burst in the deciduous forest (as defined by Borchert et al. 2005, across a range of species). Each latitudinal band in eastern North America will be sampled in a separate 10-12 day field excursion, working northwards during the excursion. Because temperature anomalies in the eastern USA/SE Canada tend to occur north-south through this region in unison (Bryson & Hare 1974), if spring is warmer or cooler than usual in one part of the region, phenology should be shifted approximately equally all across the region, still enabling valid standardized comparison.

The sites in Mexico will also all be sampled within a 7 day period. On this basis, we will sample eastern North America in 5 latitudinal bands on 5 separate field excursions, with additional sampling in eastern Mexico. The earliest sampling in North America will involve the PI and technician, the 2nd the technician only, the 3rd the PI only, the 4th the technician only, and the 5th the PI only. On all these excursions two undergraduate assistants will also be employed (rotating between two pairs of assistants on alternating trips).

Planned excursion/sampling dates, averaging the two sources of phenological estimation plus allowing for short breaks between excursions are:

- 1) Southern Florida, southernmost Georgia. 18th – 30th May.
- 2) Northern Georgia-South Carolina-North Carolina. 7th – 17th June,
- 3) Virginia-Maryland-Delaware. 20th June – 3rd July
- 4) New Jersey-southern New York State– southern New England. 6th-14th July.
- 5) Northern Vermont and New York State–Quebec. 19th July-31st July.
- 6) Veracruz, Mexico. 7th May.

Assessment of herbivory damage using Sigmascan. The percentage area loss to herbivory will be assessed using Sigmascan software on scans of the dried and pressed leaves. The leaf outline of the intact leaf will be reconstructed by eye and drawing the intact shape on the screen, and areas consumed by herbivores or fungi will be subtracted from the area of the leaf if it were intact. This approach using software and scanner minimizes subjectivity in assessing leaf area loss. Some element of subjectivity in reconstructing area of the intact leaf from a leaf badly damaged by chewing insects is always present, but we feel that for an experienced user this component of error is minor. The percentage leaf area loss will be area-adjusted on Sigmascan; larger leaves on the branches will be weighted according to their area and count more than small leaves in terms of assessing herbivory.

At the sampling height used (4-5m off the ground) in North America and in montane eastern Mexico there are no common mammalian or bird herbivores which would regularly damage leaves. The only source of herbivore damage is likely to be insects. We will categorize the herbivore damage

into five categories 1) chewing 2) skeletonizers 3) galls 4) leaf miners 5) leaf rollers. A sixth category of fungal spot damage will also be included. The categorizations will follow generally accepted classifications, such as illustrated in Cranshaw (2004) which is a work from an acknowledged academic expert in insect damage to plants. Although phloem or xylem feeding insects may be significant (Andrew & Hughes 2005) the difficulty of assessing such pinpoint damage necessitates a concentration on other more accessible types of damage. It is relevant to note however that if heavy infestations of phloem feeders (including gall forming aphids) occur, this might complicate the relevance of the results by causing premature abscission and loss of leaf area (Williams & Whitman 1986). Evidence of heavy infestation of phloem feeders on harvested branches will be noted, for possible exclusion of such sites from the final data analysis.

Consistent and reliable assessment of damaged leaf areas and types of damage requires considerable patience and experience. We have found that more ambitious computer programs for automated scanning and leaf area assessment appear to lack sophistication and often give results widely at variance with 'common sense' assessment of leaf area damage. Hence we favor combining Sigmascan with by-eye demarcation of areas using a mouse on a scanned image. Even with this technique, we have found that undergraduate workers do not tend to use sufficient care, lack the careful drawing skills and make many mistakes in categorizing leaf damage types. Additionally, multiple users will tend to introduce an unknown element of inconsistency in the results. Hence we propose to continue using a technician, Ms Mei Ling Lee (see resume), who has been trained and supervised by the PI over the past 18 months, and who has been found to carry out the job in a consistent and careful manner from the first season of leaf gathering. Ms Lee is also very experienced in tree species identification from leaves and from wild trees in the field, and in navigating between sites. Ms Lee will be supervised by the PI and refer to him for advice on problematic classifications.

Ms Lee is able to consistently categorize and assess area damage of roughly 300 leaves per day using Sigmascan, and so will be able to complete the assessment of all species and sites in sufficient time. If each species is collected from an average of 50 sites yielding 300 leaves per species per site, this gives 50 days' work per species. This gives approximately 200 days' work per year, with some room for additional site collection. Additional time during the year for the technician will be spent organizing field collecting, several weeks on actual field collecting, and organizing the pressing and preservation of the leaves.

For the sites in Mexico, local undergraduates will be employed to sample leaves according to the precise protocol used in eastern North America, which will be demonstrated by the PI on a visit to Mexico several weeks before the sampling time. Leaves will then be scanned (in groups of about 20 at a time laid on a standard office flatbed scanner) and the gif files sent by e-mail for analysis by the technician using Sigmascan.

Completely consumed leaves. Various studies have suggested that in tropical forests a significant proportion of leaves are completely eaten off by herbivores and not counted in herbivory studies (e.g. Coley 1982). Our trial year of data suggested that in temperate forests, very few leaves on forest edges at 4-5m height are completely eaten, or nearly completely eaten. Close inspection of sun branches during leaf scanning showed that petioles remaining from completely consumed leaves were extremely rare (<0.1%), and nearly-consumed leaves (more than 95% eaten) were also extremely rare (around 0.2%). Sometimes a particular branch would be heavily defoliated: this can give the impression that such defoliation is common because it is so noticeable from a distance; however random sampling shows that as a proportion of branches these are very uncommon. Even the rare badly damaged leaves (>95% eaten) showed no tendency towards yellowing or abscission but remained a healthy green color, suggesting that if severe damage does occur, abscission does not quickly 'hide' this damage from herbivory assessments. While most leaves have some insect damage,

the damage is relatively evenly dispersed among leaves. Thus we have seen no evidence to indicate that complete consumption of leaves is a major error factor in assessing site-to-site differences in herbivory. It is possible that certain localized defoliation outbreaks can be much more intense in terms of leaf damage, but our preliminary results from tens of sites fairly randomly scattered through the eastern US suggest that such severe defoliation would be highly localized and is generally uncommon on a whole-landscape scale. Even if a 'hidden' proportion of leaves were actually being completely eaten off, one could reasonably expect that in sites with more leaves completely eaten, there will also be more leaves heavily damaged but still on the tree to be counted, shifting the average strongly upwards. It is important to bear in mind that the aim of this study is to test for evidence of a latitudinal trend in leaf damage, not to provide an absolute measure of such damage. Thus, a trend in relative amount of herbivory can show up as a shift in averages regardless of whether the 'uppermost limits' of leaf damage have gone missing from the totals.

Table 2. Summary of methodology.

- Sites sampled in early summer 2007 and 2008, in 6 latitudinal 'clusters', starting in early May in southern Mexico, mid May in southern Florida, late May in central/northern Florida and working northward to eastern Canada. Phenologically standardized to allow for seasonal delay with latitude.
- Each sampling takes place along forest edges (edges of roads and tracks passing through forested areas) within forest areas >10ha in total extent.
- Only 'sun' leaves, in direct sunlight during mid morning to mid afternoon, are sampled, at 4-5m height.
- Up to four species are sampled at each site: *Q. alba*, *A. rubrum*, *F. grandifolia*, *L. styraciflua*.
- About 30 sun leaves from a randomly chosen branch from each of at least 10 individuals per species are sampled (300 leaves in total per species per site).
- Twigs are pressed and dried with leaves in place.
- Dried leaves are carefully removed and damage to each leaf assessed and categorized using Sigmascan

Analysis of results. The large number of sites scattered across a range of latitudes will allow a far more authoritative and closely standardized analysis of the biogeography of herbivory than any previous study. As well as analysis using correlation and regression against latitude and temperature variables, the use of GIS (which the PI's lab is equipped for) allows statistical analysis in relation to soil and topographical variables. Data from soil samples gathered at each site will be used, and maps of such data are available through STATSGO (2001). Percentage abundance values of each species along the edge where it is sampled will also be used as a parameter in the analyses, to allow for effects of population density of herbivore abundance. Multiple regression will also be applied to help explain spatial patterning. Having a large number (several tens) of sites for each species will allow selective elimination of particular soil or landscape types that might confound a latitudinal trend. For example, the selective analysis could exclude the shallow Appalachian fold belt soils of parts of the northern section of the transect, while leaving in those northerly sites which are on deeper soils of plains, more comparable with those to the south-eastern USA and mid-Atlantic states. This might show up trends confounded by soil differences by ensuring a relatively uniform soil background for the analysis. Interpolation surfaces of herbivory drawn using a GIS will also be instructive in understanding spatial trends in herbivory in each species. Fungal spot damage will also be analysed in relation to herbivory, and for any latitudinal trend in itself.

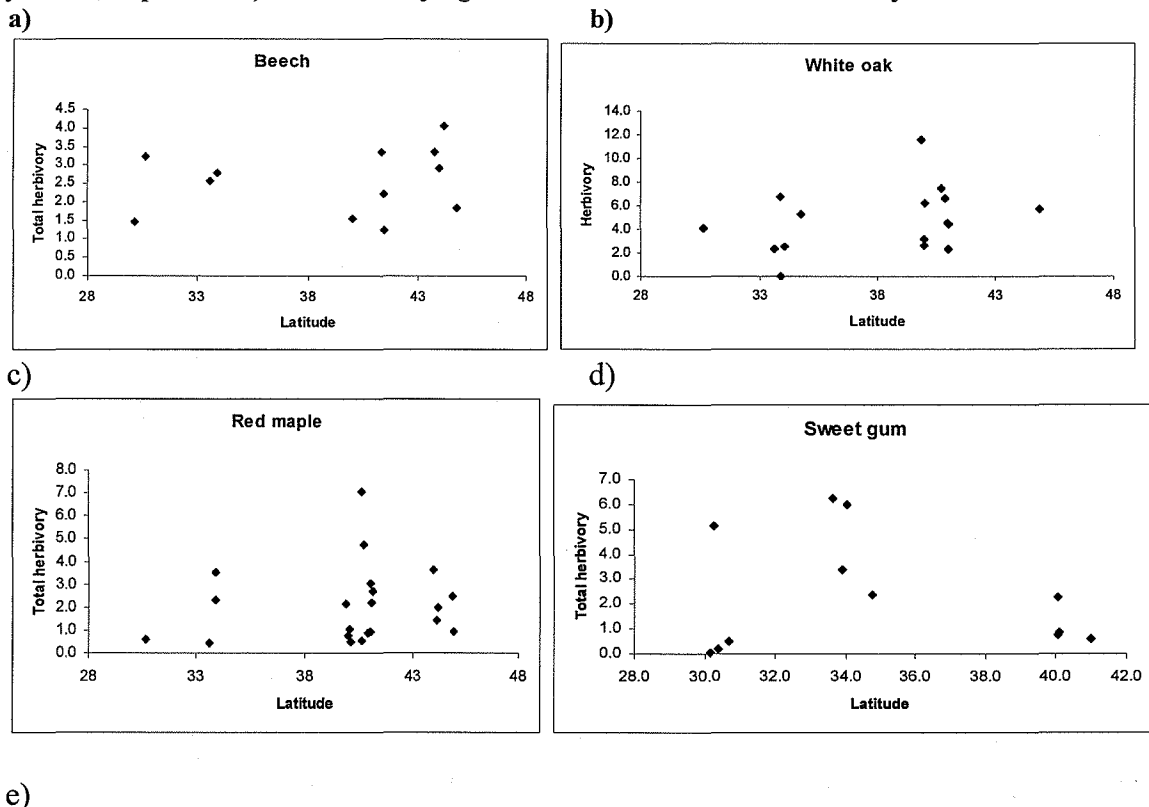
The two years of herbivory data gathered in this study will be analysed separately for any trend which might show up in one year and not the next, due to the effects on insect populations of year-to-year climatic variation or other factors.

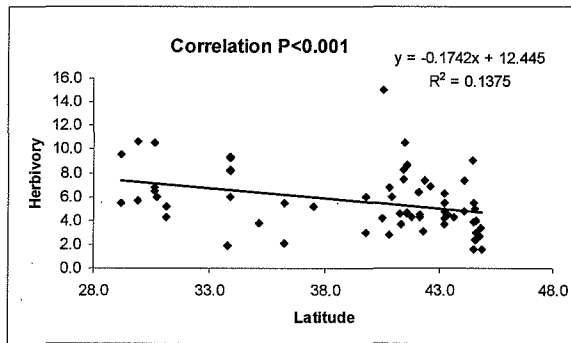
ANTICIPATED OUTCOMES and BROADER IMPACTS. This study will provide some of the first standardized, systematically gathered data to help answer one of the 'big' questions in ecology; are plants at lower latitudes (in warmer and relatively frost-free climates) subject to more

herbivore attack? Only two smaller scale studies on individual plant species, involving rather less thorough geographical coverage, have attempted to answer this question (Swihart et al. 1994, Andrews & Hughes 2005). The proposed study will offer a considerable leap forward in understanding the biogeography of herbivory, and provide a more informed basis for discussion of the many aspects of community and ecosystem functioning which follow on from this (Coley and Barone 1996).

A preliminary first year of data for this study in summer 2005 (Fig.4) revealed no statistical evidence of greater herbivory towards low latitudes within eastern North America, within species. Analysis of the results showed that latitude (and the temperature parameters such as mean annual temperature and winter temperature which are likely to be involved in producing any latitudinal trend) explain a very small proportion of the variance. It is interesting to note that the study by Andrew and Hughes (2005) in Australia also found no significant latitudinal trend in herbivory. However, our preliminary study involved 1) a small number of sites, and 2) fewer leaves sampled per species per site (75 leaves, as opposed to 300 per site in the proposed study). The smaller sample sizes may have introduced additional scatter in the data that could have confounded any latitudinal pattern. Regardless of whether a pattern is apparent or not, it is essential that this fundamental hypothesis is systematically tested. Interestingly, whole-community level (not species-specific) latitudinal study by the PI on freshly fallen autumn leaves *did* show a significant latitudinal trend (Fig 4e) in herbivory damage. It may then simply be that more sites and greater numbers of leaves per site are necessary to show whatever trend is present.

Fig 4. a-d (below) Results from a preliminary round of early summer collecting of leaves in 2005. Total herbivory area percentage lost, vs latitude. Each data point is one site. Counterclockwise: a) *Fagus*, b) *Quercus alba*, c) *Acer rubrum*, d) *Liquidambar styraciflua* Bottom right 4e) summary of results from recently fallen autumn leaves in 2005 from mixed species sites in eastern North America, at the whole community level (study by the PI; unpublished). A statistically significant latitudinal trend in herbivory is indicated.





If additional years of data, and much more thorough sampling, corroborate the pattern found in sampling from summer 2005 (namely that there is no significant difference between amounts of herbivory at different latitudes in the eastern North American region), what would be the implications for community and ecosystem functioning? The mechanism by which trees from climates with colder winters are supposed to suffer less herbivory depends fundamentally on the view that temperate zone insect populations are less tightly controlled by density dependence, because their populations are always recovering from the 'knock back' of winter (e.g. Dobzansky 1950). In warmer climates, by contrast, populations are regarded as being more stable and likely limited by food supply, or by predation. If there is no difference, it would seem to imply that density dependence is about as important throughout the temperate forest biome, regardless of temperature/latitude. This has further knock-on implications for the likelihood of populations at a range of trophic levels to be involved in niche specialization, coevolution and host specificity (*sensu* Dobzansky 1950), with implications for the hypotheses regarding mechanisms behind species richness and other trends (Janzen 1972).

If the 'expected' gradient in herbivory is not found in nature, a complicating factor is the possibility that herbivory might only be kept at a relatively low level in warmer climates (despite the absence of a strong dormant season) by more abundant chemical and physical defenses in the leaves (Coley and Barbone 1996). For instance, long-lived tropical leaves are certainly more physically robust than the soft deciduous leaves of the temperate zone. Coley & Barone (1996) have also suggested that tropical leaves have a higher percentage of condensed tannins than temperate leaves, at around 5.5% in tropical leaves as opposed to 1.9% in temperate leaves. However, the exceptionally high levels of tannins found in many samples of temperate tree leaves (upwards of 40%; Forkner et al. 2004) seem to put this in some question. Follow-on studies from the present project might attempt standardized latitudinal comparisons of tannin content within several widespread North America tree species, to understand if there is indeed any latitudinal trend in defenses.

Dry seasons in the lowland tropics are associated with lower insect population levels (e.g. Lowman 1985), and this might tend to complicate any trend seen between the warmest parts of the eastern USA and eastern Mexico. However, the winter dry season in the mountains of eastern Mexico is relatively weak, and biological activity of many plants and insects, plus decay of leaf litter, continues year-round (G. Williams-Linera pers. comm.).

Leaf lifetime of temperate broadleaf species (even within the same species) may differ by several months between the ends of the latitudinal gradient sampled here. This study will provide a perspective only on herbivory accumulated during the earlier part of the growing season. It is possible that additional herbivory will occur at different rates between different latitudes before the end of the growing season. However, most herbivory seems to accumulate in the early part of the season so it is not likely that this is a major confounding factor (results summarized by Coley &

Barone 1996).

Leaf damage by insect herbivory is of course only one of the various forms of herbivory which can affect plants. Xylem and phloem feeding, both above and below ground, is another form that will not be quantified by this study. Mammalian herbivory near ground level is another. Essentially, the range of potential plant-herbivore interactions is very broad and it is necessary to define particular limits for what to study in a consistent manner. An all-encompassing study of herbivory would have to limit itself to very few sites and fail to give the broad biogeographical perspective needed. It is also important to note that this study focuses on the empirical effects of insect damage on leaves, not on the taxa which produce the damage (although evident 'outbreaks' of caterpillars or phloem feeders on the branches will be noted in each site description). Again, a focus on identifying the precise types of herbivores present would preclude broad geographical data gathering at a large number of sites; this study will concentrate on the plants' perspective.

The relative isolation of beech populations in eastern Mexico might potentially affect insect herbivore diversity on this species, and through this the amount of herbivory. By analogy to island biogeography, herbivores may be more likely to go extinct, and less likely to colonize, scattered stands of beech. 'Isolation' and small regional range sizes have been hypothesized to affect insect herbivory levels on other widespread plants (e.g. *Pteridium*; Shuter & Westoby 1982). However, *Fagus* is very abundant (dominant) where it does occur in the mountains of eastern Mexico, with genetic analyses revealing the lack of severe genetic bottlenecks during glacials (Rowden et al. 2004), so this island effect might not be significant. *Liquidambar* is very widespread and often dominant throughout the eastern mountains of Mexico, so is unlikely to have suffered much depletion of its herbivore fauna due to island biogeography.

It is important to note that a study such as this cannot directly translate into a quantification of carbon turnover or other biogeochemical aspects of the ecosystem because of the way in which leaves are sampled. The sun leaves sampled are only part of the volume of the canopy. The present project is, then, a comparison of community functioning using consistent standards, not a biogeochemical study.

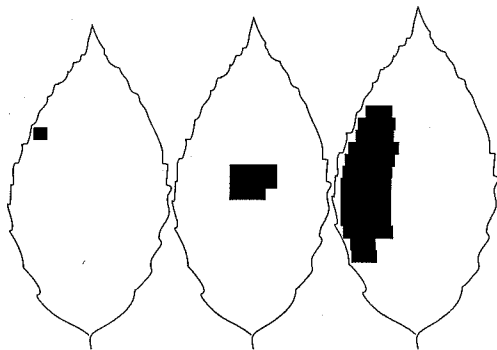
If a latitudinal gradient in herbivory at the community or intraspecific level is found by this study, it will be of fundamental importance in community ecology in confirming what was previously suspected based on non-standardized results. On the other hand, if no such gradient is found it will play an important role in breaking myths and putting community ecology on a firmer footing.

The findings of this study are also potentially important in understanding the future behavior of communities in response to climate change (IPCC 2001). If warming occurs, will insect damage to wild forest communities be greater? This might have implications for the survival of certain tree populations or even species, especially if level of secondary defensive compounds in leaves is finely adjusted to latitudinal gradients in herbivory (e.g. Salgado and Pennings 2005).

Building upon the findings of this two-year study, we intend to expand this work in future years to other geographical areas to understand if similar trends (or lack of trends) occur in different regions. These possible future studies include: latitudinal variation in herbivory and other leaf characteristics in forests from northern to southern China and Malaysia. Eastern Asia offers a continuous forest gradient from temperate through paratropical to tropical rain forest, and standardized studies on particular species or the whole community would be of considerable interest along this broad latitudinal gradient. It is hoped that separate additional work will be possible on latitudinal variation in fungal spot damage which will also be recorded by this study (and possible co-variation with levels of herbivory damage).

Educational outcomes. The fieldwork will provide training for at least eight undergraduate researchers from North America's most minority-diverse school, Rutgers Newark, in field ecological and analytical methods. The core of the field work, including site location, identification and leaf harvesting, will be conducted by a number of undergraduate students under close supervision of the PI and technician. The field experience gained by undergraduates at Inecol in Mexico (in association with Dr Williams-Linera, see attached letter) will also be valuable. South-eastern Mexico is traditionally an under-privileged area of Mexico.

Outreach activities will include a Website presenting the philosophy and findings of the project to grade school children, linked to other ecological educational resource sites on the Web (e.g. The Classroom Earth site: <http://www.classroomearth.org/about/>). An interactive teaching resource based upon the methodologies for assessing leaf area damage will be prepared and made available for teachers of grade school children. The resource page will present samples of different insect damage types, simplified theory of plant-herbivore interactions, plus 'templates' for each of the four species being studied, showing of different areas lost to herbivory damage (see below), so that damage can be assessed easily by eye. Diagrams of 'damage types' (e.g. leaf miners) will also be presented. Schools from different locations in the USA, Canada and Mexico will be contacted and invited to contribute their own findings to a combined table of results, and these will be summarized by the PI and presented as maps and other diagrams for the participants to understand how their own region fits into the geographical picture of herbivory. The work will develop the students' eye for looking at nature, for understanding ecological processes, and fitting the local into the broader picture. It is hoped that the time series of results over several years obtained by school students can contribute to scientific understanding of spatial and temporal variability in herbivory. This method will also be developed for undergraduate classes by the PI (some initial trial classes have proven to be enjoyable and instructive for students), and presented in ecological teaching forums at conferences such as ESA, and on Web-based undergraduate teaching forums.



(left) a few examples of an extensive selection of 'leaf damage assessment templates' produced by the PI for teaching purposes that can be used by students to assess herbivory damage percentage, for a nationwide science study. These examples are for *Fagus*, but it is possible to produce such templates for any leaf type. From left to right, 0.5%, 5%, 20%. The templates are printed and held up next to a leaf to assess damage and provide rapid subjective assessments. While such judgements do not rival Sigmascan assessment for accuracy, from initial trials with undergraduate classes by the PI it seems that there is a strong general consistency in results between different individuals and students in different colleges.