INVESTIGATION OF AN EPIDEMIC OF BALSAM POPLAR LEAF BLOTCH MINER, *PHYLLONORICTER NIPIGON,* (LEPTIDOPTERA: GRACILLERIIDAE) IN THUNDER BAY, ONTARIO

by

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ABSTRACT

In the summer of 2018, an outbreak of *Phylonorycter nipigon*, commonly known as the balsam poplar leaf blotch miner, was identified on the Lakehead University campus in Thunder Bay, Ontario. All balsam poplar and trembling aspen trees in the study area were affected. Leaf blotch mines created by the larvae of this species were present on nearly 100% of leaves, thereby prompting this study.

This study investigated leaf blotch mine counts and frequency distributions, leaf surface areas and two methods of measuring them. It was determined that leaf blotch mine frequency distributions follow a poisson-like pattern, and that a linear relationship exists between leaf surface area (cm^2) and number of leaf blotch mines present on a leaf. Although balsam poplar has a significantly higher average leaf area (cm^2) than trembling aspen, its leaves did not have a significantly larger average number of leaf blotch mines.

ACKNOWLEDGEMENTS

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INTRODUCTION

An epidemic of *Phyllonorycter nipigon* (Freeman), known by the common name balsam poplar leaf blotch miner, occurred in the Thunder bay area in the summer of 2018. *P. nipigon* is a lepidopteran insect belonging to the family Gracillariidae. Larvae feed between the upper and lower epidermis of leaves on trees belonging to the Salicaceae family - most commonly balsam poplar (*Populus balsamifera)* and trembling aspen (*Populus tremuloides*) (Davis 2001). The full extent of the outbreak of *P. nipigon* in Northwestern Ontario has yet to be determined, though during the summer of 2018 it was observed that nearly 100% of balsam poplar and trembling aspen trees < 20 m in height displayed leaf blotch mines produced by this insect. The affected area included the Lakehead University Campus in Thunder Bay, Ontario and was observed as far west as Dryden, Ontario, and reaching south to the U.S border (Henne, D. personal observation).

Through counting blotches - or mines - on randomly selected leaves it was determined whether or not a relationship exists between leaf surface area (cm^2) and the number of leaf blotch mines present on a leaf. Distribution of leaf blotch mines on both balsam poplar and trembling aspen were investigated, comparing tree species leaf areas and number of mines present on samples collected. Tools used to measure the surface area of irregular shapes were evaluated for accuracy and effectiveness for the purpose of leaf area measurement and relationship to number of leaf blotch mines.

LITERATURE REVIEW

HOSTS

Balsam poplar, *Populus balsamifera*, and trembling aspen, *Populus tremuloides*, are two species of trees commonly found across North America and in the boreal forest of Ontario (Perala 1990, Zasada 1990). The wood of these trees is used in light frame construction, boxes, crates and pallets, as well as veneer, plywood and other engineered wood products. As such, it is important to understand potential threats to these tree species and what effects these threats might have on forest stands and wood supply. It is known that several species of leaf blotch miner are present in the boreal forest and feed on balsam poplar, trembling aspen, and other members of the Salicaceae family (Hopkin 1996).

LEAF MINERS

Generally, a leaf miner is the larva of an insect that lives in and feeds on the tissue between the upper and lower epidermis of leaves. These larvae most commonly belong to the order Lepidoptera (moths and butterflies), though other known leaf miners can be sawflies (Symphyta - Hymenoptera), common flies (Diptera) and some beetles (Coleoptera) (Cranshaw 1993). Damage caused from these insects is commonly referred to as a mine – which is the feeding channel inside the parenchyma or epidermis tissue of a leaf belonging to the host plant. In these feeding channels the outer walls will remain undamaged, shutting off the mine from the outside and providing both a feeding and living area for the larva (Hering 1951). Leaf mines can fall under two categories: linear mines (ophionome mine), and blotch mines (stigmatonome mine). Larva of species creating a linear mine will move and feed in one direction only, continually moving forwards (Figure 1). In contrast, blotch mining larvae will feed in several directions with no apparent system, creating a large 'blotch' that covers this excavated area (Hering 1951).

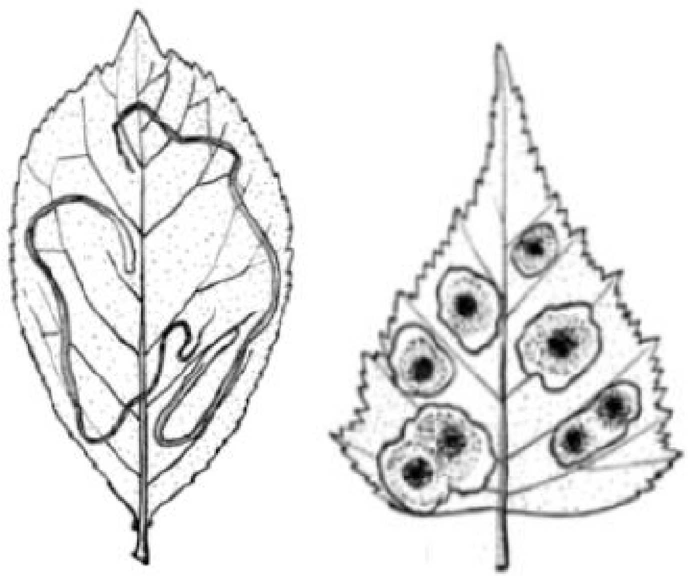
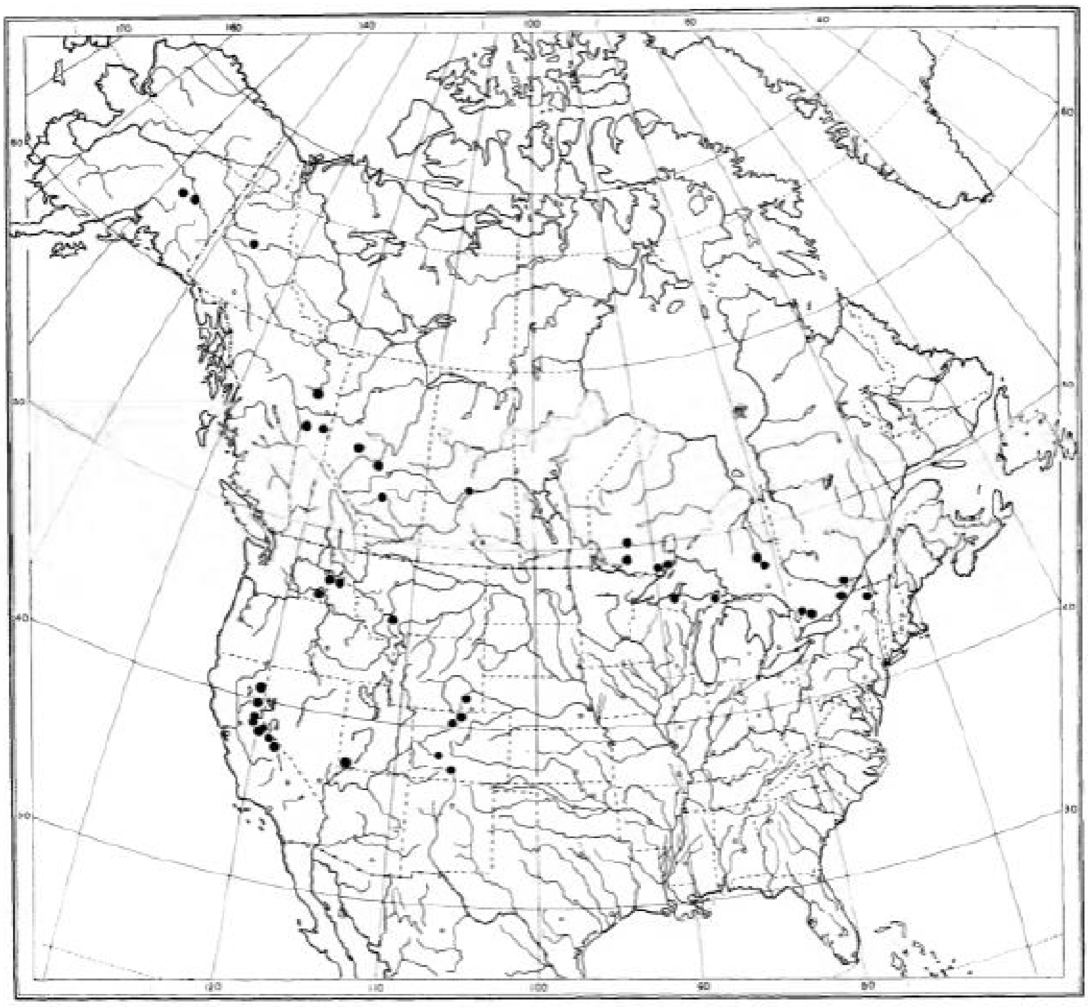


Figure 1 - Linear vs blotch mines Source: Hering (1951)

The Genus *Phyllonorycter*

The poplar leaf blotch miners in question belong to the genus *Phyllonorycter*. Members of this genus produce blotch mines and are present across much of northwestern Ontario. For example, *P. salicifolela, P. apparella,* and *P. nipigon* are a few that can be found in our area (Biggs 1995). Historical outbreaks by *Phyllonorycter* spp. leafminers in Ontario were reviewed in annual documents compiled by Natural

Resources Canada and the Canadian Forest Service. These forest health reports are compiled from annual surveys and published the following year. Forest health condition reports suggest that almost every year there is an outbreak of leafminers in Northwestern Ontario, though these outbreaks may vary in size (Biggs 2002).



Source: Davis (2001)

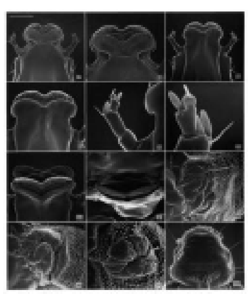
Figure 2 - Distribution of P. nipigon in North America

Phyllonorycter nipigon

The balsam poplar leaf blotch miner, *P. nipigon* was identified as the species responsible for widespread damage on poplar trees in the summer of 2018. Adult females of this insect deposit eggs in the epidermis of a leaf belonging to the host species (either balsam poplar or trembling aspen) where the larva feeds, eventually creating a blotch type mine. This mine serves as a home for the larva, providing both food and shelter. The larva will pupate inside the mine and emerge as an adult moth (Davis 2001). Originally discovered in 1970 and described as *Lithocolletis nipigon* (Freeman), *P. nipigon* is a tiny moth with wings roughly 4 mm long. *P. nipigon* is widely distributed across North America, from Ontario to Alaska, and as far south as Colorado and the Sierra Nevada Mountains in California. The range of *P. nipigon* in Ontario is generally west of the Nipigon River (Figure 2).

A detailed description of the external anatomy and taxonomically important structures of *P. nipigon* was compiled by Davis (2001) and is summarized below. Eggs laid by adult female moths are roughly 0.324 mm long, 0.228 mm wide and 0.12 mm deep. Upper surfaces of eggs are reticulate and lacking micropapillae. Edges have a slightly less developed circumferential fringe and a maximum width of 0.28 mm. The eggs will have 2-4 micropyles and are surrounded by 9-10 cellular partitions (Davis 2001). *Phyllonorycter* species have been found to lay between 11-33 eggs per female (Bagdavaze 1963), with some averaging as much as 102 eggs (Baumgartner 1981). It has been suggested that this variation could result from the availability of carbohydrates to ovipositing females (LeRoux 1971).

After the eggs hatch there are several instars that are categorized as either sap feeding (instars 1-3) or tissue feeding (instars 4-5). Sap feeding instars have strongly depressed bodies with a maximum length of 3.4 mm and width of 0.6 mm. Here, the larvae can be described as having a head with a maximum width of 0.3 mm, greatly depressed and triangular. Setae are less present and 3 pairs of stemma are present in a widely spread lateral row. Six pairs of cranial setae remain, with one pair dorsally and five pairs laterally. The labrum is bilobed and less than 0.4 of the width of the head with a serrated margin containing 7-9 serrations per lobed. Mandibles are large and flat with three elongated acute cusps and one small cusp. The labium is described as smooth with the anterior margin excavate at middle and the spinneret is absent, as are the maxillary and labial palpi. The hypopharynx is broad and densely covered with small spines along the anterior margin, with the margin being slightly incised at the middle. Antennae are reduced, 3-segmented with numerous short basiconic sensilla (Davis 2001). Bodies of sap feeding instars (1-3) generally lack setae except for the lateral to dorsal and ventral plates. Legs, prolegs, and crochets are absent and paired ambulatory callosities can be found both dorsally and ventrically (Figure 3).



Source: Davis (2001)

Figure 3 - P. nipigon larva at third instar (sap feeding): 94, head, dorsal view (60 um); 95, labrum, dorsal view (38 um); 96, head, ventral view (60 um); 98, antenna, ventral view (20 um); 99, antenna, dorsal view (8.6 um); 100, anteroventral view of mouthparts (38 um); 101, anterior view of mouthparts (38 um); 102, dorsal ambulatory callosity of prothorax (30 um); 103, ventral ambulatory callosity of prothorax (27 um); 104, ambulatory callosity of abdominal sternum 4 (17.6 um); 105, abdominal segments 9, 10, dorsal view (86 um).

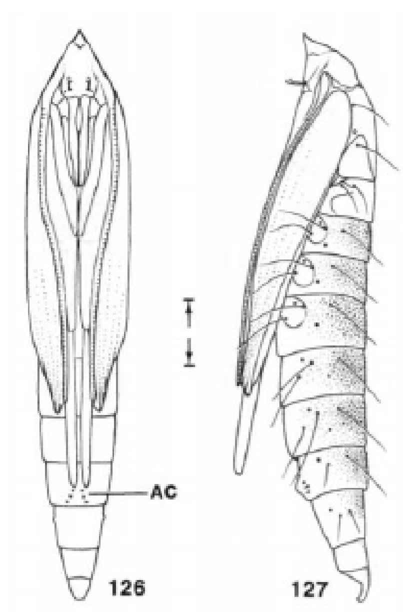
Tissue feeding instars (4-5) have heads with a maximum width of 0.4 mm. Heads are almost round with full mouthparts present. The frons is elongate and roughly 0.85x the distance to the epicranial notch. The ecdysial line terminates near epicranial notch. Chaetotaxy is relatively complete and all three setae are present. Three stemma are present and antennae are moderately long. There are three pairs of epipharyngeal spines present, lateral spine slightly reduced, with several secondary spines covering the inner ventral perimeter of the labrum. The mandible has three large median cusps, along with one smaller lateral and one smaller mesal cusp. There is one single mandibular seta present. The spinneret is a relatively short and stout tube with fleshy, strongly bifurcate lobe arising from ventral apex. The labial palpus has a relatively long basal segment bearing one short sensillum and a small globose apical segment bearing a longer sensillum apically (Davis 2001).

Source: Davis (2001)

Figure 4 – P. nipigon larva at fifth instar (tissue feeding): : 106, head, dorsal view (75 um); 107, ventral view of head, thoracic segments 1,2 (0.27 mm); 108, head, ventral view (120 um); 109, detail of labium and hypopharyngeal spines in Figure 108 (75 um); 110, labial palpi and spinneret (25 um); 111, antenna, anterodorsal view (13.6 um); 112, maxilla, anteroventral view (15 um); 113, proleg of abdominal segment 4 (30 um); 114, abdominal segments 9, 10, dorsal view (176 um); 115, lateral view of Figure 114 (200 um); 116, ventral view of Figure 114 (120 um); 117, anal proleg (30 um).

The thorax of tissue-feeding instars (4-5) is described as having an unpigmented indistinct, and relatively smooth pronotal plate with legs well developed, having widely spread and separated coxae. The pretarsal claw is strongly curved and has a relatively large and blunt axillary spine. The abdomen has triangular dark brown and rugose dorsal plates with 19 pairs of primary setae. Crochets with one or two anterior rows of 5-7 hooks and a single posterior row of 5-6 hooks. The anal plate has four pairs of setae, and anal crochets containing 30-34 hooks arranged in two irregular, circular rows (Davis 2001).

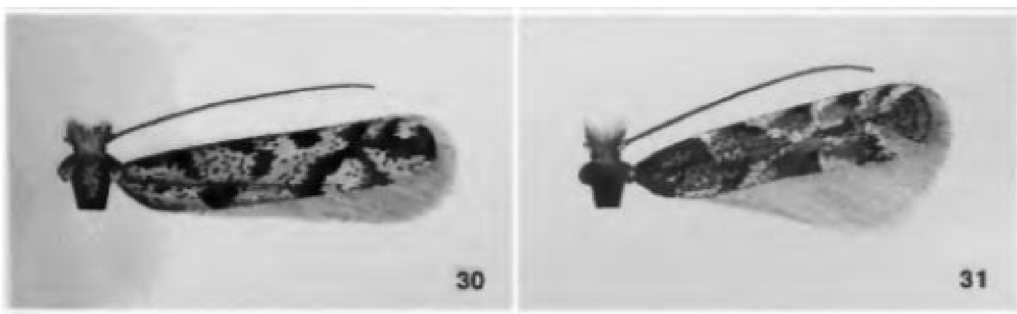
Pupae (Figure 5) reach a maximum length of 5 mm and a width of 0.9 mm. The vertex has a triangular cocoon cutter. The forewings and antennae extend to the caudal margin of abdominal section A5. Hindleg extends to A7. Dorsum of A2-A8 is almost completely covered with densely pack short and scattered spines.



Source: Davis (2001)

Figure 5 - P. nipigon pupa: 126, ventral view (0.5 mm); 127, lateral view (AC = accessory cremaster, scale: 0.5mm)

Adult moths (Figure 6) have forewings measuring 3.4-4.4 mm in length. Moths are small and slender with reddish brown forewings having 4-5 highly variable white bands. Male genitalia have symmetrical valvae that gradually taper to a nearly straight, slightly downcurved acuminate apex. The head has a rough vertex with a mixture of white and brown piliform scales. The frons is smooth with broad white scales that are heavily suffused with brown. The antennae are grey with a single row of scales encircling each segment. The length of antennae are roughly 0.8x that of the forewing. The labial palpus is mostly white and light to dark brown laterally (Davis 2001).

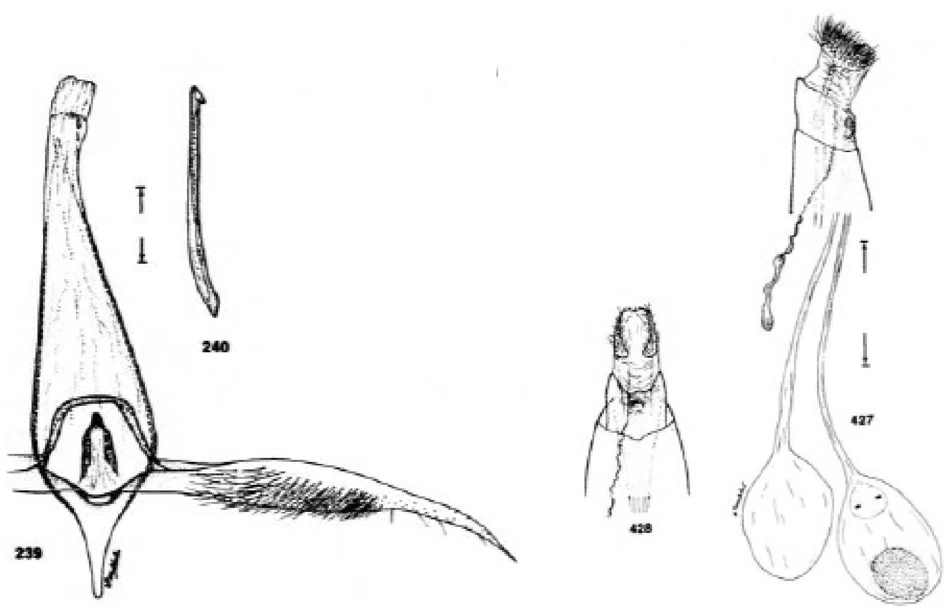


Source: Davis (2001)

Figure 6 - P. nipigon adult forewings; 30, 4.3 mm in length; 31, 3.7 mm in length.

The thorax of the adult moth has a mostly white dorsum, speckled with dark brown tipped scales. Venter is white and the forewing is variable – pale reddish to bronzy brown and usually with five white bands extending across wings that fuse with four broader white dorsal bands. Dorsal bands are reduced and usually separated by reddish brown bands. The white bands are usually speckled with dark brown. The wing fringe is pale grey, and the hindwing and fringe are uniformly pale gray as well. The legs are dark greyish brown dorsally, white ventrally, and the apices of tibial and tarsal segments are slightly lighter in colour (Davis 2001).

The abdomen is greyish brown dorsally and white ventrally. Any conspicuous sex scaling is absent. The eighth sternum of males is elongate and gradually tapers caudally to an acute apex. Male genitalia (Figure 7) have a slender vinculum tapering to a moderately elongate saccus. Transtilla are slender. Valva is symmetrical, elongate and slender, tapering to a nearly straight and downcurved acuminate apex. The aedeagus is slender with a small subabical lobe and is relatively short – 0.65-0.7x the length of the sternum. Female genitalia can be described as having an accessory bursa with an elongate duct equalling the length of ductus bursae and arising from a short and slightly enlarged common duct (antrum) immediately anterior to the ostium. There is a common duct with a small and narrow sclerotized ring. The signum is a single elliptical to pyriform lightly sclerotized disk bearing a single pair of minute papillae (Davis 2001).

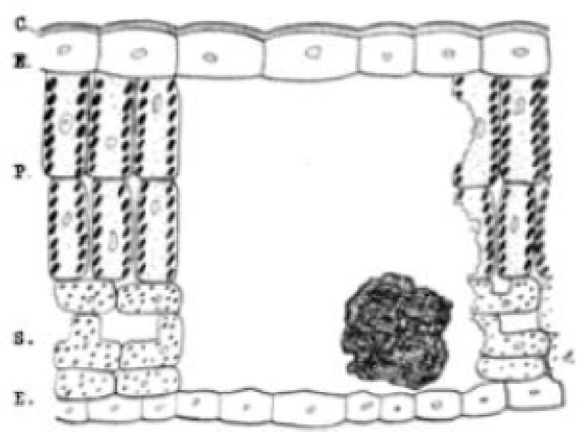


(Davis 2001)

Figure 7 - P. nipigon: male and female genitalia. 239, full view, male; 240, aedoeagus; 427, lateral view, female; 428, lateral view, female.

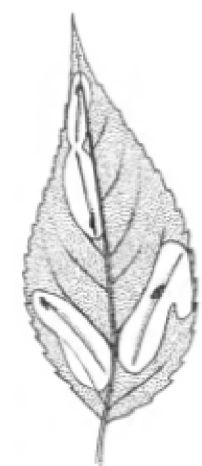
Damage

Phyllonorycter species create full depth blotch mines between the upper and lower layers of leaves belonging to the host tree. Figure 8 shows the anatomy of a full depth mine on a leaf. A cross section of a leaf can be split into four layers: cuticle, epidermis, palisade parenchyma, and spongy parenchyma. Palisade parenchyma is found just under the upper surface or cuticle of a leaf, below the epidermis, and is made of tightly packed cylindrically shaped cells to which it owes its name. Below the palisade parenchyma and just inside the outer surface of the leaf lies a layer of loosely packed cells called the spongy parenchyma. Full depth blotch mines created by the Gracilariidae (Figure 9), which includes more specifically the genus *Phyllonorycter*, begin within the epidermal layer of a leaf and later progress to the parenchyma (Hering 1951).



Source: Hering (1951)

Figure 8 - Cross section of a full depth mine. (C - cuticle, E- epidermis, P - palisade parenchyma, S - Spongy parenchyma)



Source: Davis (2001)

Figure 9 - Leafmines of *P. nipigon* on balsam poplar

Potential relationships between leaf area and the number of eggs laid on the surface of the leaf have been suggested for leaf-mining moths that create linear mines *Lithocolletis quercus* and *Stilbosis quadricustatella*, finding that both species tend to lay eggs on leaves with larger surface areas. In the case of *L. quercus*, however, leaf area explained only 9-29% of variation in mine density (Auerbach 1989). Also of note is that in the case of *S. quadricustatella* larger leaves were found to have more mines than smaller leaves on average, though there was no correlation between distributions of leaf size between trees and mine densities (Simberloff 1987). The same was found for another study on *Scolioneura betuli*, also leaf miner, where it was found that there was a higher number of larvae on leaves with larger leaf areas (Tuomi 1981).

METHODS

LEAF SAMPLING AND MEASUREMENTS

This study began with the collection of sets of leaves from infested trees for inspection of leaf blotch mines. Leaves were haphazardly collected from balsam poplar and trembling aspen trees less than 10 m in height on the Lakehead University Campus on August 15th, 2018. Leaves were placed in Ziploc bags, labelled, and then frozen for later inspection. Initially, 100 leaf samples were selected from each of 5 different trees – 2 trembling aspen and 3 balsam poplar. These first 500 leaf samples were then assessed for the number of leaf blotch mines per leaf. The resulting leaf blotch counts were then entered into a database so that different statistical analyses could be performed. Examining leaves of each tree species, and then determining the number of leaf botch mines per leaf, the 100 leaf samples per tree produced data that would then be manipulated to determine if there were any differences between the number of leaf blotch mines on a balsam poplar or a trembling aspen.

The potential relationship between leaf surface area and the number of leaf blotches per leaf was investigated based on examination of 50 leaf samples from one of the balsam poplar trees and 50 leaf samples from one of the trembling aspen trees. Leaf area was measured using two tools: an online service called SketchAndCalc and an image processing program (ImageJ) designed for scientific analysis of multidimensional images.

SKETCH AND CALC

To measure leaf area using SketchAndCalc leaves were placed on a lightboard and photographed alongside a measuring tape for scale (Figure 10).

Figure 10 - balsam poplar leaf sample against lightboard next to measure used for scale

Images were then uploaded to the SketchAndCalc service where the measuring tape was used as a reference to assign a scale to the image (Figure 11).

Figure 11 - assigning scale to SketchAndCalc

From here, leaf margins were traced using the free draw too. Once completed a surface area was generated for that leaf (Figure 12).

Figure 12 - leaf margin has been traced and surface area calculated

This process was repeated for each of the 50 leaves selected for both the balsam poplar and trembling aspen sample trees. Results would then be compared to those achieved through ImageJ software for the same leaves.

IMAGEJ

ImageJ, an open source, free image processing program (https://imagej.nih.gov/ij/) was used next. A new set of images was taken of the same set of leaves to be used for processing and measurement through ImageJ. The result of re-taking the images for a second measurement was a shuffle of the leaves and as such the leaves are the same, but leaf numbers were assigned differently among the samples. The new set of images was taken using a scanner on a white background with a circular sticker measuring .635 cm in the corner for a scale reference (Figure 13). Each leaf was placed on the same background and scanned, producing a catalogue of all 50 leaf samples for each balsam poplar and trembling aspen.

Figure 13 - balsam poplar sample scanned with calibrated 0.635 cm 'dot' in corner

The image was opened in ImageJ and converted to 8-bit for analysis (Figure 14).

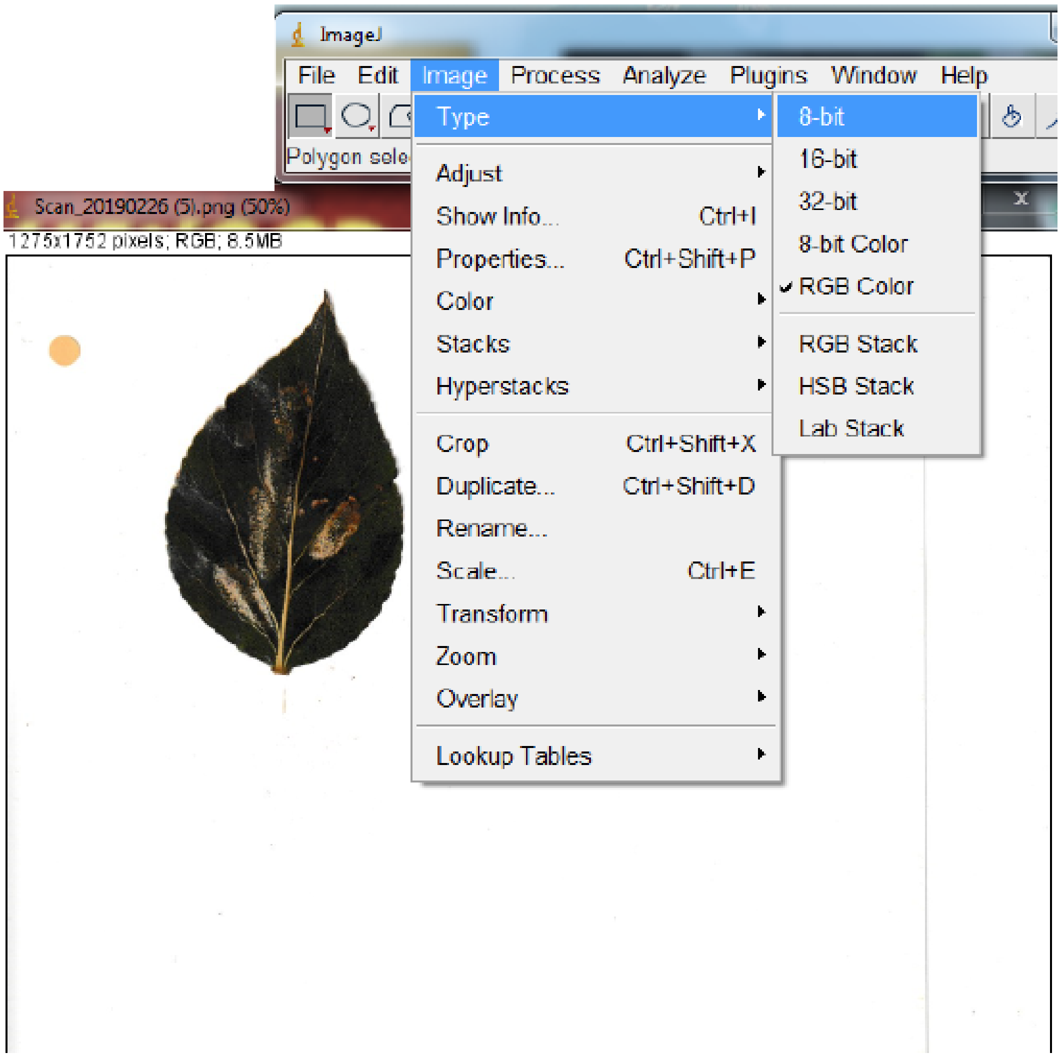


Figure 14 - converting to 8-bit in ImageJ

Zooming in on the scale dot, the straight line tool was used to measure from the farthest visible pixel on either side (Figure 15). Using the Analyze tab and the Set Scale function the distance measured is given in pixels - in this case the dot is 38 pixels wide and a known distance of 0.635 cm can be assigned.

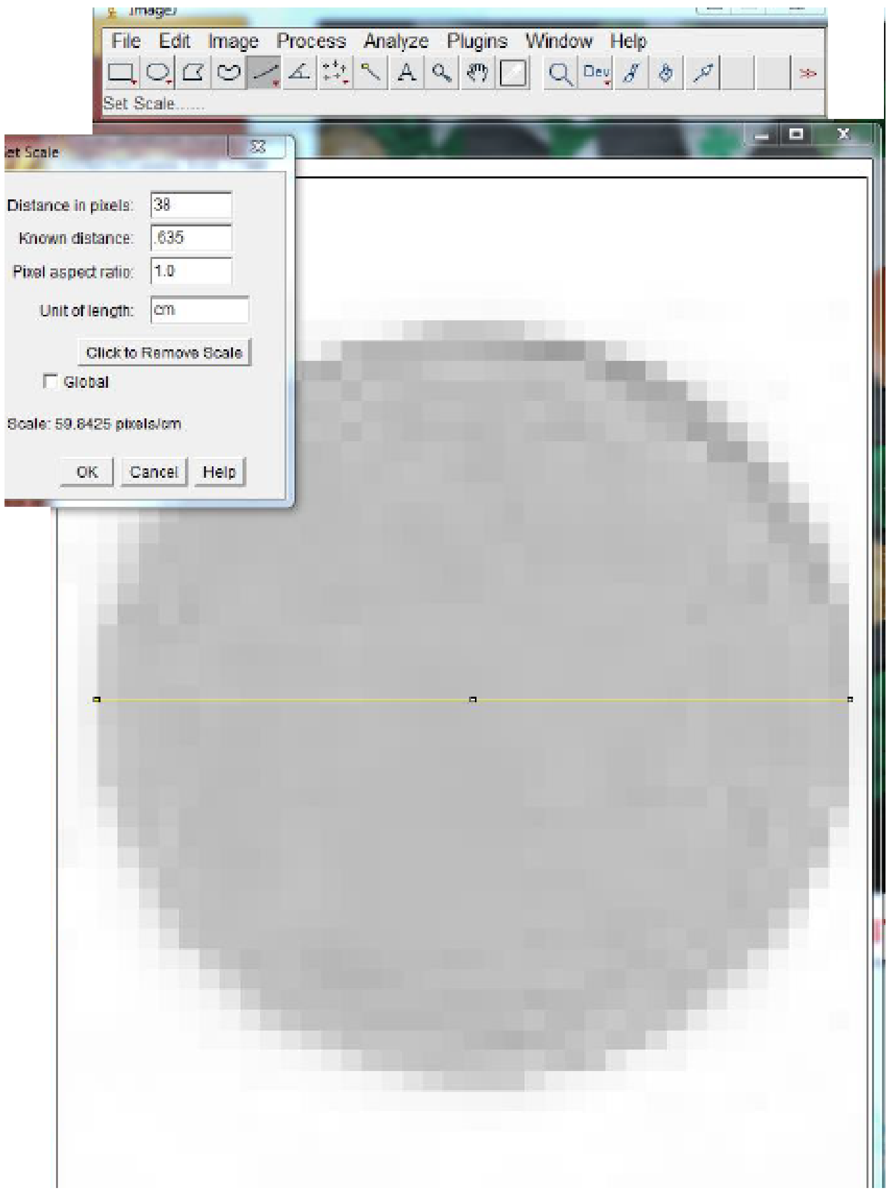


Figure 15 - using the scale dot to assign a scale to the image

Next, using the Edit tab and Options, colours were set to make the background white and the foreground black. Under the Image tab and Adjust, the Threshold function was selected and default values were changed using the automatic setting. Automatic setting was selected to avoid any bias in image processing by the user. Once the image has had the threshold balanced, all shadows are eliminated from the leaf margin and the results were a black and white outline of the leaf area (Figure 16).

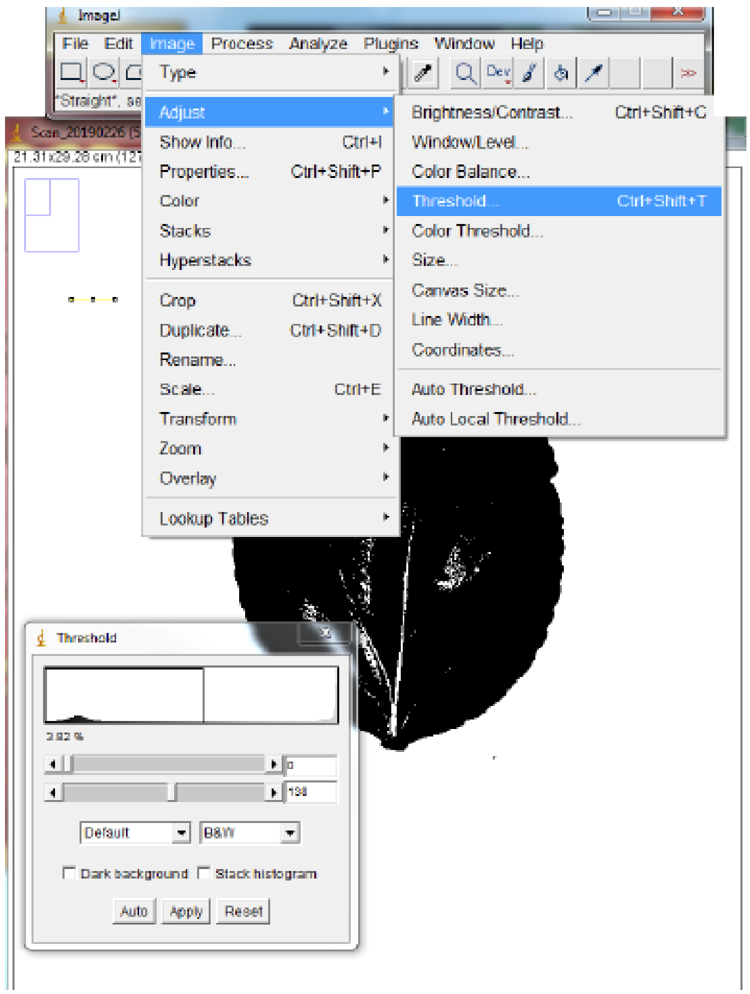


Figure 16 - adjusting image threshold in ImageJ

Next, using the magic wand tool, the leaf is selected and margins are highlighted yellow. Under the Edit tab, the fill tool is used and the leaf is filled in (Figure 17).

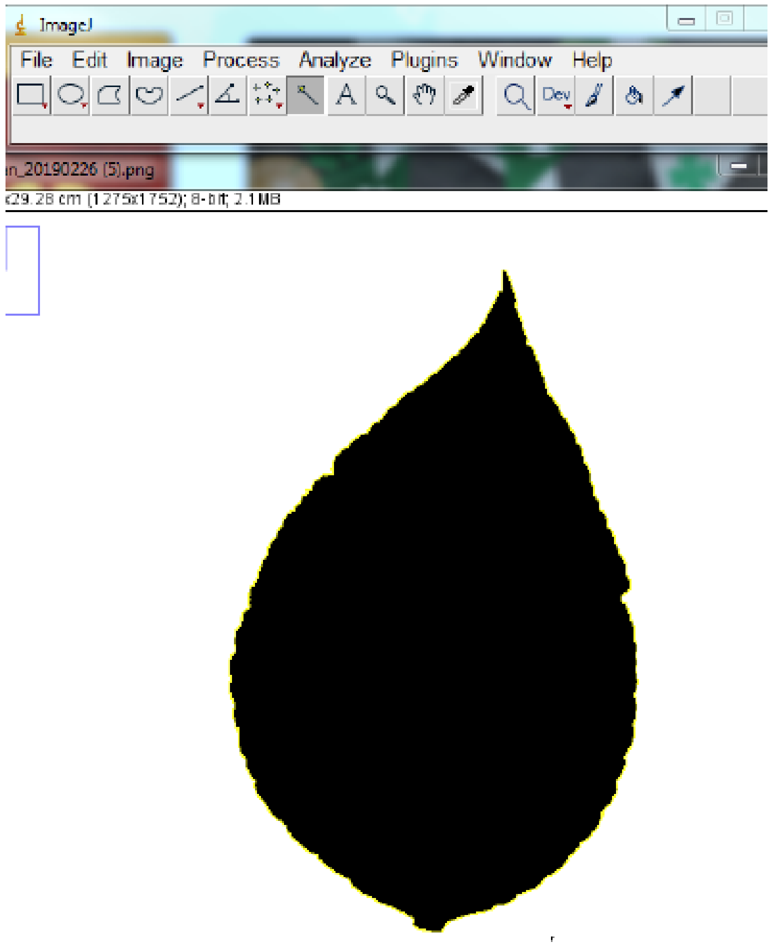


Figure 17 - selected image has been filled using both magic wand and fill tools

The final step in this process is using the Analyze tab and clicking Measure. In the case of this leaf a surface area of 24.432 cm^2 has been calculated (Figure 18).

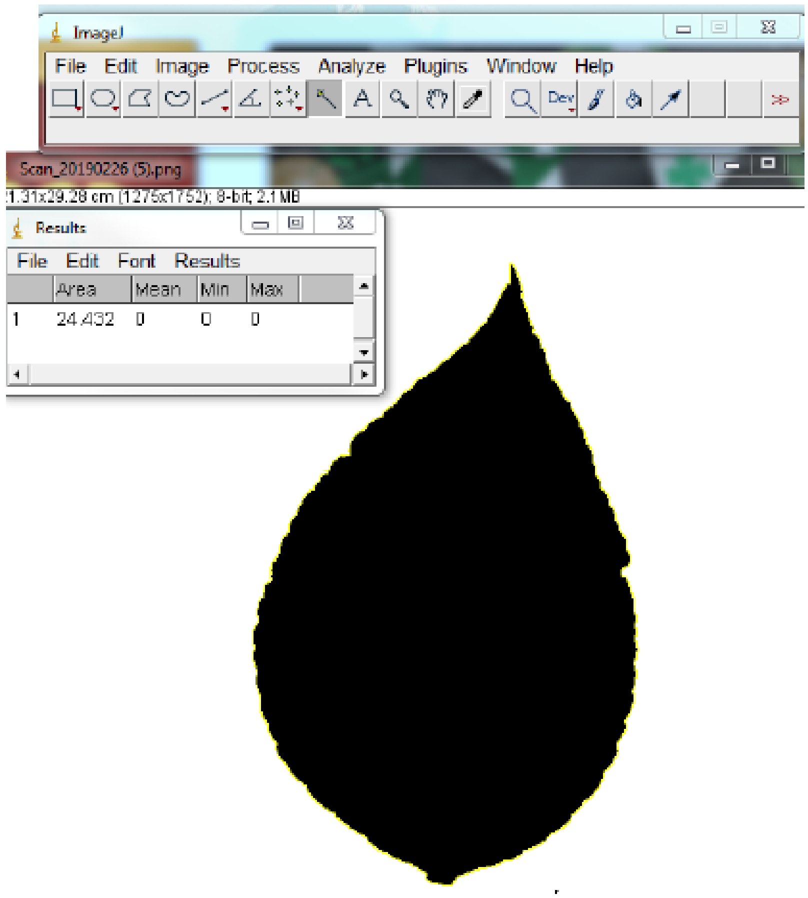


Figure 18 - leaf area measured at 24.432 cm^2

Again, this process was repeated for the 50 leaves in each set for the selected balsam poplar and trembling aspen trees.

STATISTICAL ANALYSES

All data was entered into Microsoft Excel for statistical analysis to be performed; determining the distribution of leaf blotch mines in both balsam poplar and trembling aspen, if there is a difference in the number of leaf blotch mines between balsam poplar and trembling aspen, if there is a linear relationship between leaf surface area (cm^2) and the number of leaf blotch mines present, and if one of the tools used to measure leaf surface area (cm^2) is better suited to this application than the other.

Frequency distributions for the number of leaf blotch mines per leaf were generated for the initial 500 leaf samples, looking at the occurrence of the number of leaf blotch mines per leaf across all sample trees. Regression analyses were then performed comparing leaf surface area (cm^2) and the log-transformed number of leaf blotch mines present for the 100 sample leaves that had been used for leaf area analysis, using both ImageJ and SketchAndCalc.

T-tests (two-tailed, $t_{0.025}$) were used to test for the occurrence of significant differences in the: i) average number of mines per leaf on balsam poplar and trembling aspen, , ii) leaf surface area of balsam poplar and trembling aspen, and iii) leaf surface area measured using ImageJ and SketchAndCalc softwares.

RESULTS

Results of the investigation into leaf blotch mine distribution across 2 trembling aspen trees and 3 balsam poplar trees, involving 100 leaves sampled from each subject tree, found that there was an average of 4.41 mines per leaf for balsam poplar and an average of 3.93 mines per leaf for trembling aspen. A t-test using the log-transformed data from the 100-leaf sets of two balsam poplar and two trembling aspen trees yielded no significant difference in the average number of mines per leaf on either tree species (H_0: no significant difference between number of mines between tree species, $t_{0.025}$ df = 199, t=-1.36, p = 0.18, FTR H_0).

Table 1 - t-test of average leaf blotch mine count for both tree species

Null Hypothesis: No difference in average number of leaf blotch mines on each tree species

t-test: Paired Two Sample for Means

	Trembling Aspen	*Balsam Poplar*
Mean	0.655185617	0.6821441
Variance	0.036734975	0.048867025
Observations	200	200
Pearson Correlation	0.082378477	
Hypothesized Mean [	0	
df	199	
t Stat	-1.35969032	
P(T<=t) one-tail	0.08773326	
t Critical one-tail	1.652546746	
P(T<=t) two-tail	0.17546652	
t Critical two-tail	1.971956544	

P = 0.1754 > 0.025
t<tCrit = FTR
No significant difference

The frequency distribution of the occurrence of leaf blotch mines (1,2,3 mines etc.) on all trembling aspens and all balsam poplars was generated and it was found that the number of attacks followed a poisson distribution (Figures 19-25).

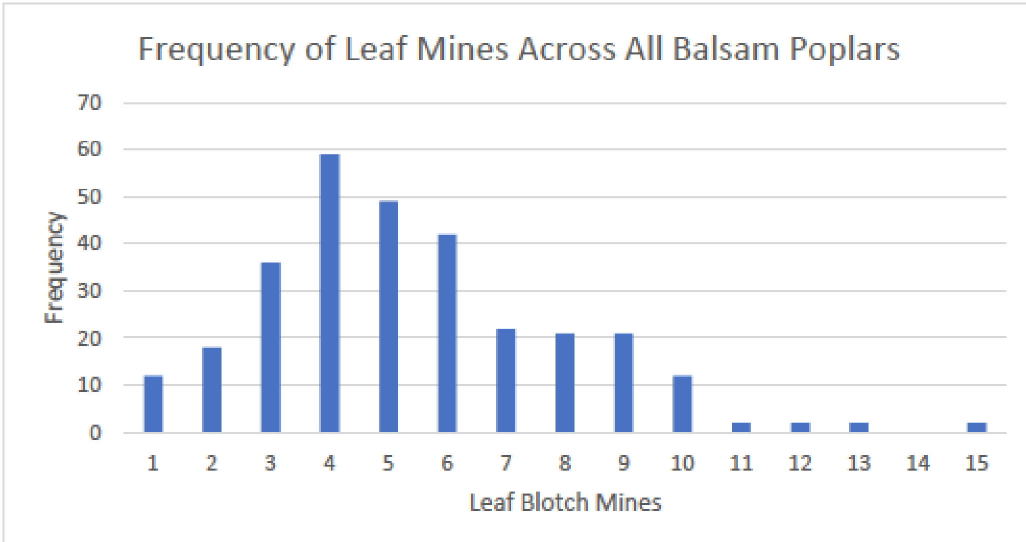


Figure 19 - histogram displaying the frequency of the number of leaf blotch mines across all balsam poplar samples

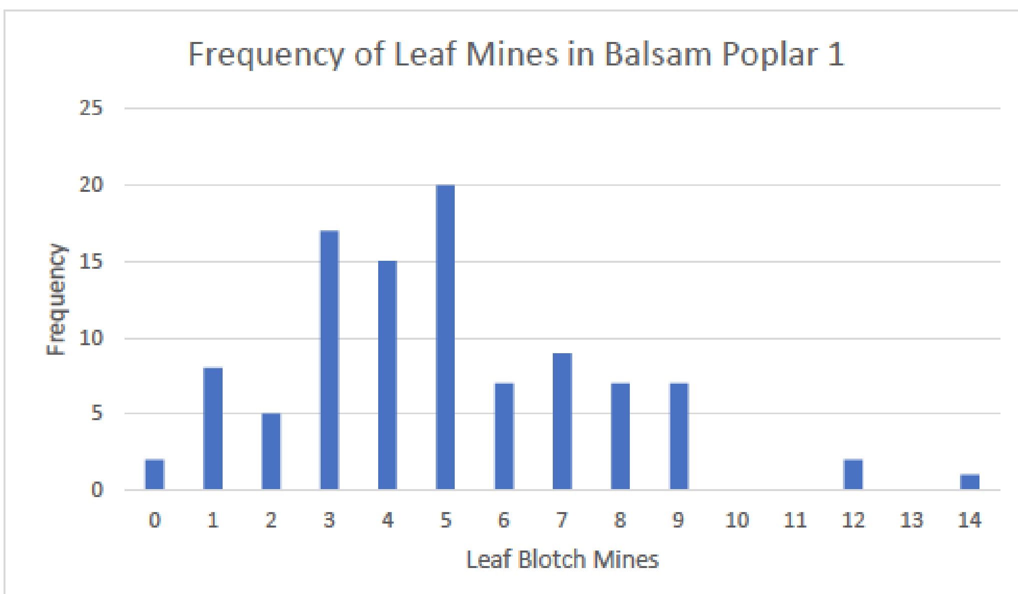


Figure 20 - histogram displaying the frequency of the number of leaf blotch mines in balsam poplar sample 1

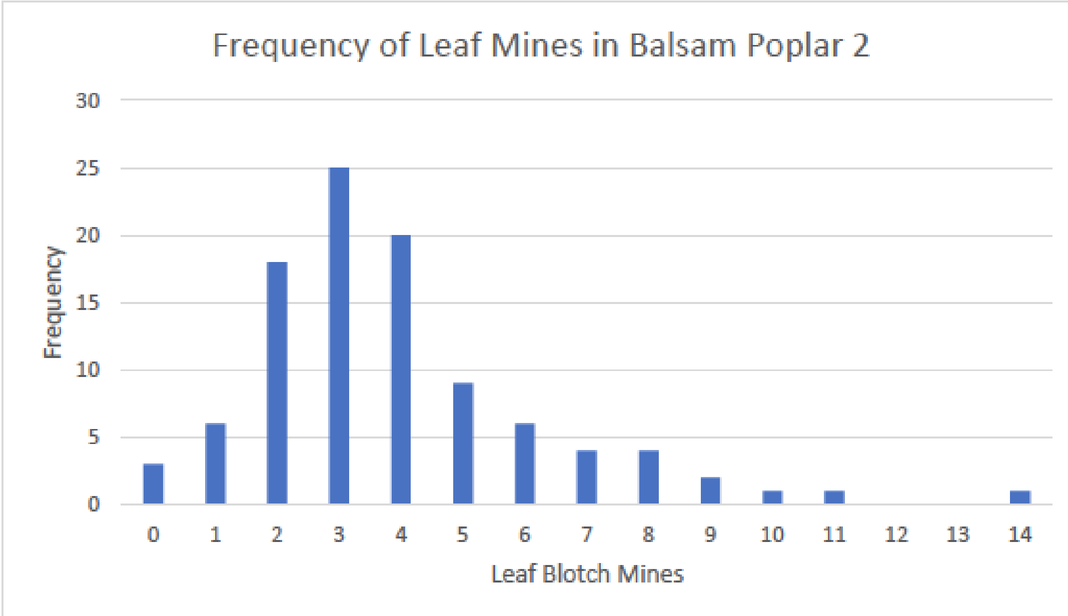


Figure 21 - histogram displaying the frequency of the number of leaf blotch mines in balsam poplar sample 2

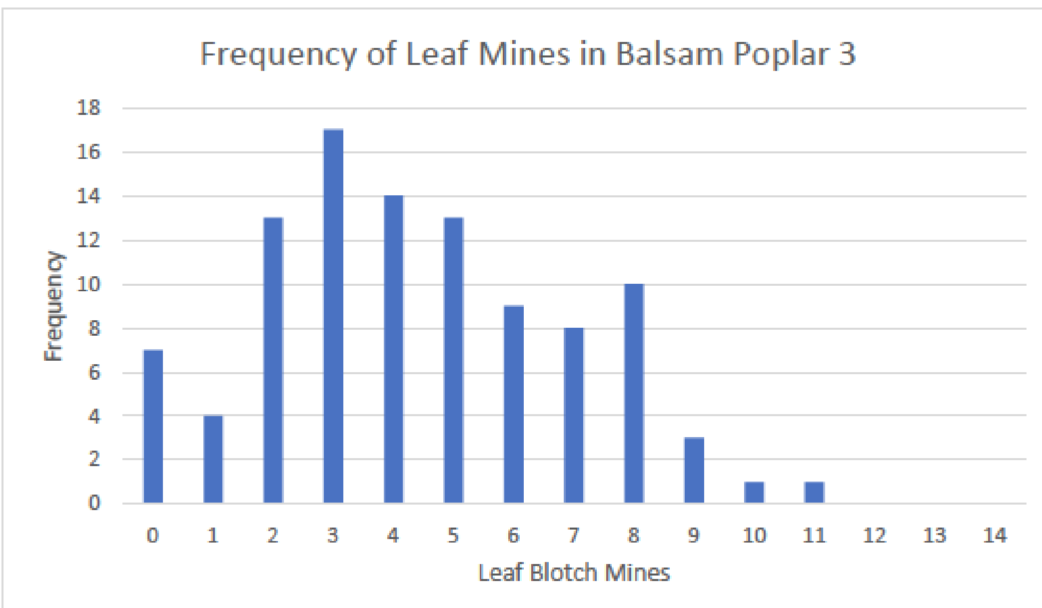


Figure 22 - histogram displaying the frequency of the number of leaf blotch mines in balsam poplar sample 3

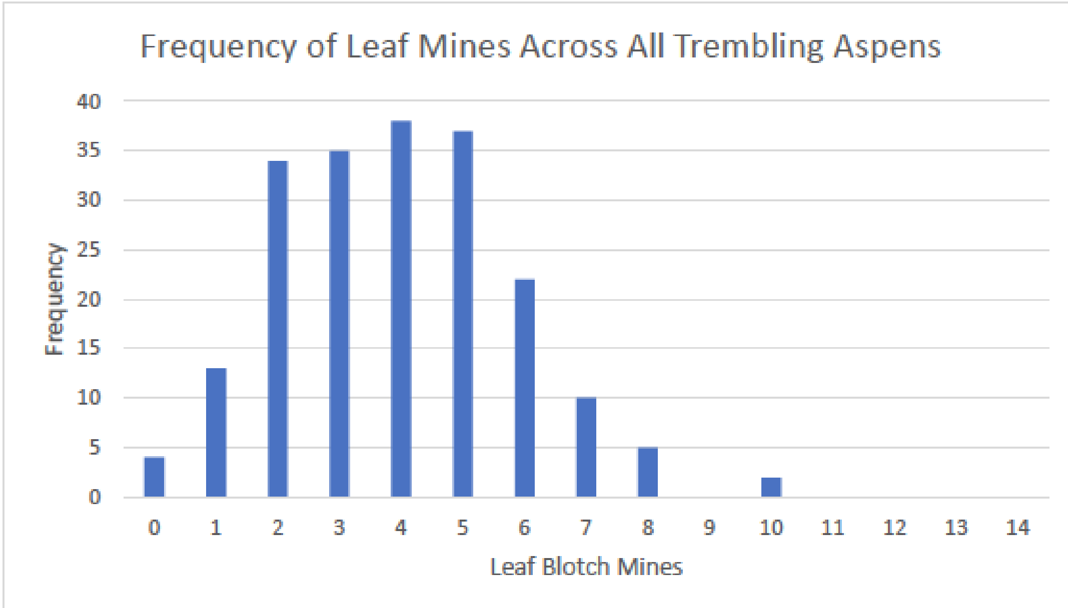


Figure 23 - histogram displaying the frequency of the number of leaf blotch mines across all trembling aspen samples

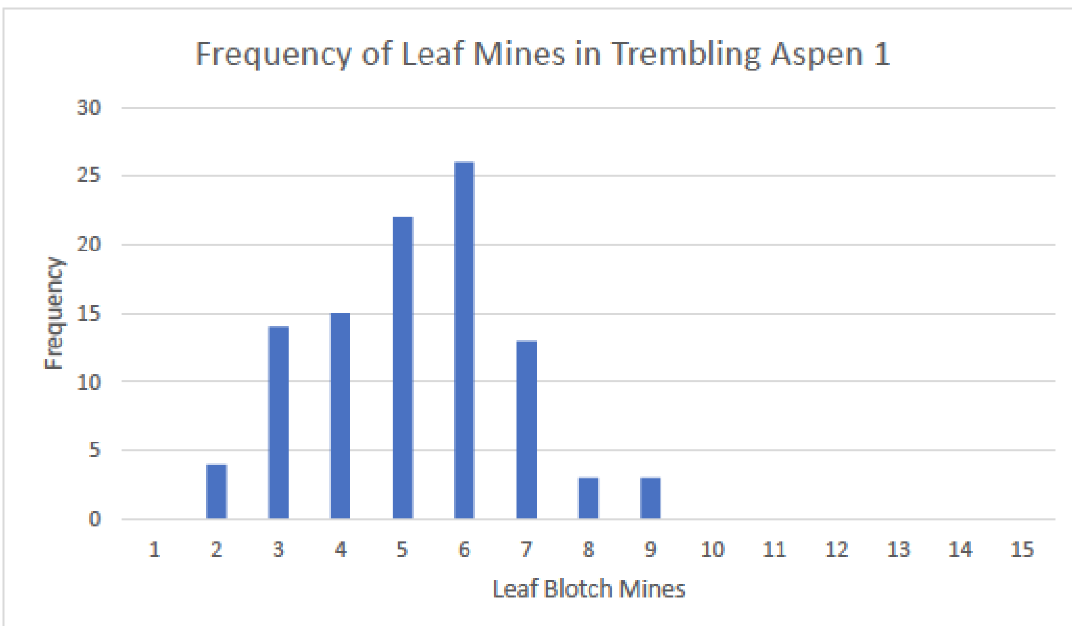


Figure 24 - histogram displaying the frequency of the number of leaf blotch mines in trembling aspen sample 1

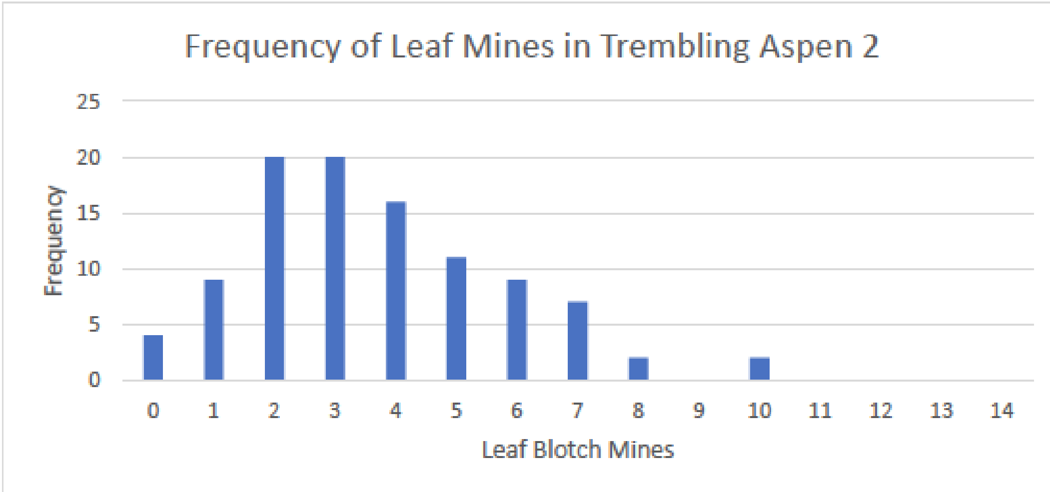


Figure 25 - histogram displaying the frequency of the number of mines leaf blotch in trembling aspen sample 2

Using the online service SketchAndCalc it was found that the average leaf area for balsam poplar trees was 25.43 cm^2, compared to an average area of 12.05 cm^2 for trembling aspen. When a t-test was performed a significant difference was found in leaf areas between the two tree species (H_0: no significant difference between leaf areas of either species, $t_{0.025}$ df =49, t = 8.5, $p < 0.00001$, Reject H_0).

Table 2 - t-test of average leaf surface area (cm2) in balsam poplar and trembling aspen - SketchAndCalc

Null Hypothesis: no significant difference between leaf areas (cm2) in balsam poplar and trembling aspen
t-test: Paired Two Sample for Means

	Balsam Poplar	*Trembling Aspen*
Mean	25.4284	12.049
Variance	74.18145861	23.76752347
Observations	50	50
Pearson Correlation	-0.308347358	
Hypothesized Mean Difference	0	
df	49	
t Stat	8.501289727	
P(T<=t) one-tail	1.65222E-11	
t Critical one-tail	1.676550893	
P(T<=t) two-tail	3.30444E-11	
t Critical two-tail	2.009575237	

P < 0.00001 < 0.025
t>tCrit = REJECT
Significant difference

A regression analysis was performed using the data of leaf surface area and the number of leaf blotch mines present per leaf for all 50 leaves each of balsam poplar and trembling aspen. A linear relationship was found between leaf area and the number of leaf blotch mines for both tree species (Figures 26, 27).

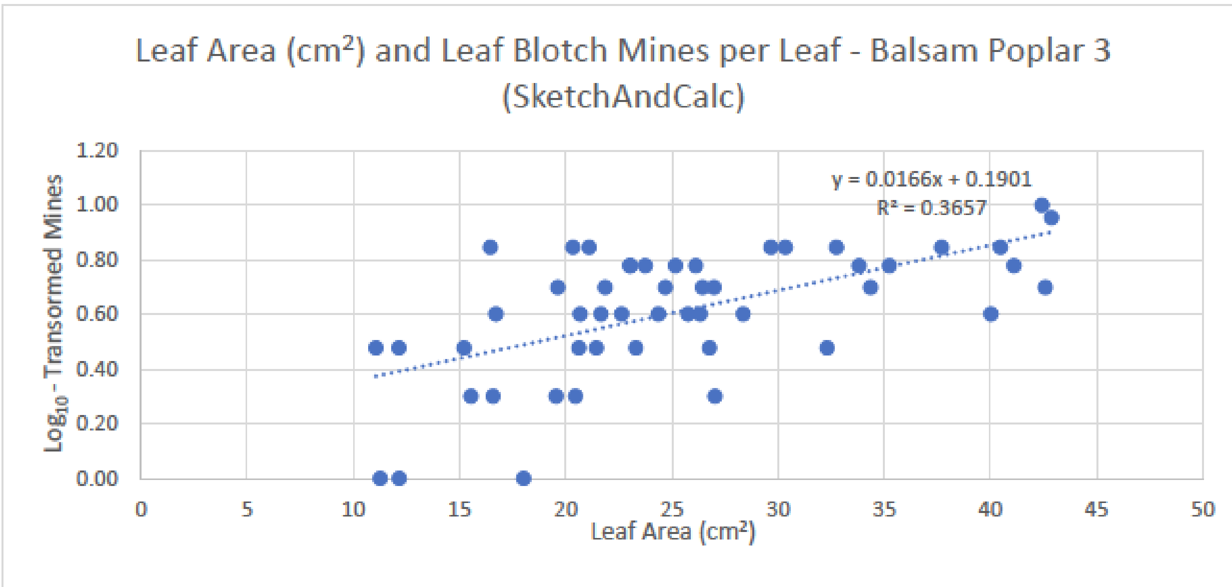


Figure 26 - scatter plot displaying leaf area (SketchAndCalc) and the number of mines per leaf for balsam poplar 3, number of mines per leaf has been log transformed

Table 3 - regression statistics: balsam poplar leaf blotch mines per leaf and leaf surface area (cm^2) - SketchAndCalc

Regression Statistics	
Multiple R	0.604708371
R Square	0.365672215
Adjusted R Square	0.352457052
Standard Error	0.189930242
Observations	50

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.998177343	0.998177	27.67066	3.30414E-06
Residual	48	1.73152785	0.036073		
Total	49	2.729705193			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.190090587	0.084489713	2.249867	0.029079	0.020212633	0.359968542	0.020212633	0.359968542
X Variable 1	0.016571359	0.003150274	5.260291	3.3E-06	0.010237308	0.022905411	0.010237308	0.022905411

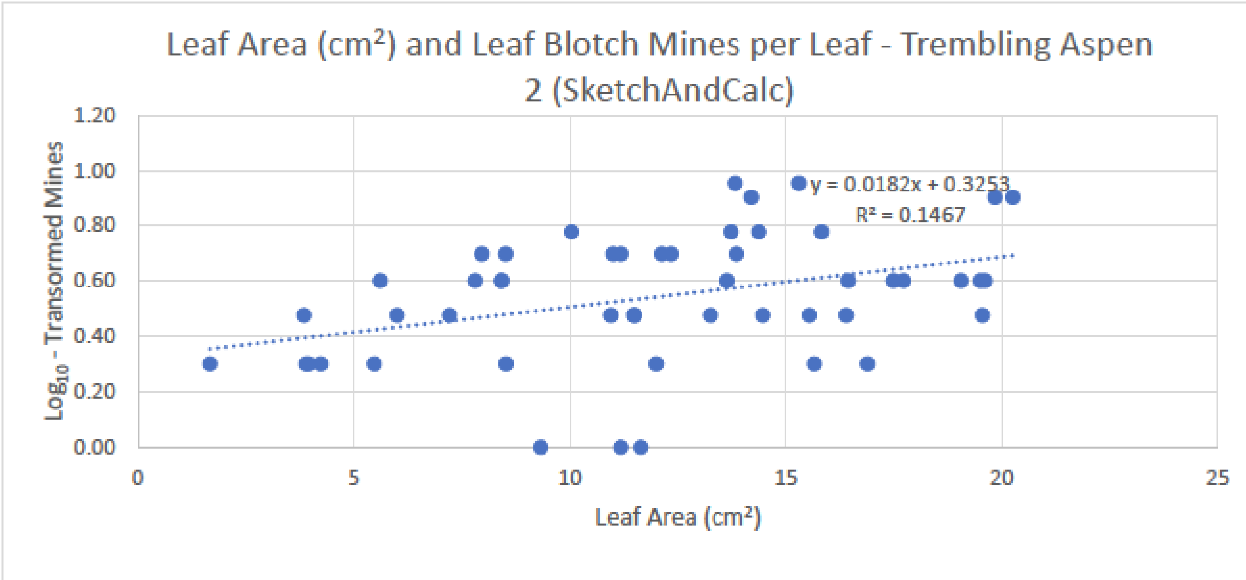


Figure 27 - scatter plot displaying leaf area (SketchAndCalc) and the number of mines per leaf for trembling aspen 2, number of mines per leaf has been log transformed

Table 4 - regression statistics: trembling aspen leaf blotch mines per leaf and leaf surface area (cm^2) - SketchAndCalc

Regression Statistics	
Multiple R	0.383052878
R Square	0.146729507
Adjusted R Square	0.128953039
Standard Error	0.215656425
Observations	50

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.383881137	0.383881	8.254143	0.00603875
Residual	48	2.232369295	0.046508		
Total	49	2.616250432			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.32531257	0.082022768	3.966125	0.000243	0.160394741	0.490230399	0.160394741	0.490230399
X Variable 1	0.018155507	0.00631935	2.873002	0.006039	0.005449603	0.030861411	0.005449603	0.030861411

The second method of leaf area measurement, using the program ImageJ, found similar results. Using the same leaves sampled for the SketchAndCalc analysis it was again found that balsam poplar had a significantly larger average leaf area than trembling aspen, with balsam poplar having an average area of 24.26 cm^2 compared to 11.16 cm^2 for trembling aspen (H_0: no significant difference between leaf areas of either species, $t_{0.025}$ df = 49, t = 8.5, p < 0.00001, Reject H_0).

Table 5 - t-test of average leaf surface area (cm2) in balsam poplar and trembling aspen - ImageJ

Null Hypothesis: no significant difference between leaf areas (cm2) in balsam poplar and trembling aspen
t-Test: Paired Two Sample for Means

	Balsam Poplar	*Trembling Aspen*
Mean	24.25798	11.1601
Variance	67.35444639	20.68932964
Observations	50	50
Pearson Correlation	-0.001815008	
Hypothesized Mean Difference	0	
df	49	
t Stat	9.862856749	
P(T<=t) one-tail	1.58875E-13	
t Critical one-tail	1.676550893	
P(T<=t) two-tail	3.1775E-13	
t Critical two-tail	2.009575237	

P < 0.00001   < 0.025
t>tCrit = REJECT
Significant difference

A regression analysis was preformed using the data of leaf surface area and the number of mines present per leaf for 50 leaves each balsam poplar and trembling aspen. A linear relationship was found between leaf area and the number of leaf blotch mines for both tree species (Figures 28, 29).

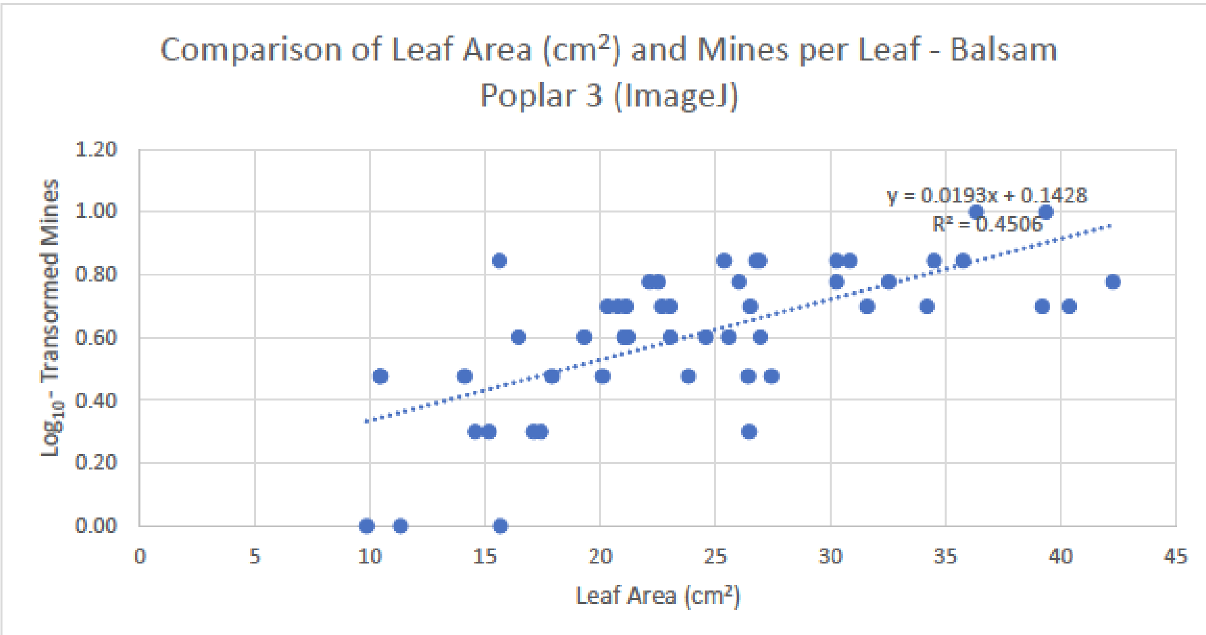


Figure 28 - scatter plot displaying leaf area (ImageJ) and the number of mines per leaf for balsam poplar 3, number of mines per leaf has been log transformed

Table 6 - Regression statistics: balsam poplar leaf blotch mines per leaf and leaf surface area (cm2) - ImageJ

Regression Statistics	
Multiple R	0.671278243
R Square	0.450614479
Adjusted R Square	0.439168947
Standard Error	0.176781705
Observations	50

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.230392865	1.230393	39.37034	9.5318E-08
Residual	48	1.500085011	0.031252		
Total	49	2.730477876			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.142783052	0.078722102	1.813761	0.075968	-0.015498343	0.301064448	-0.015498343	0.301064448
X Variable 1	0.019308153	0.003077203	6.274579	9.53E-08	0.013121022	0.025495284	0.013121022	0.025495284

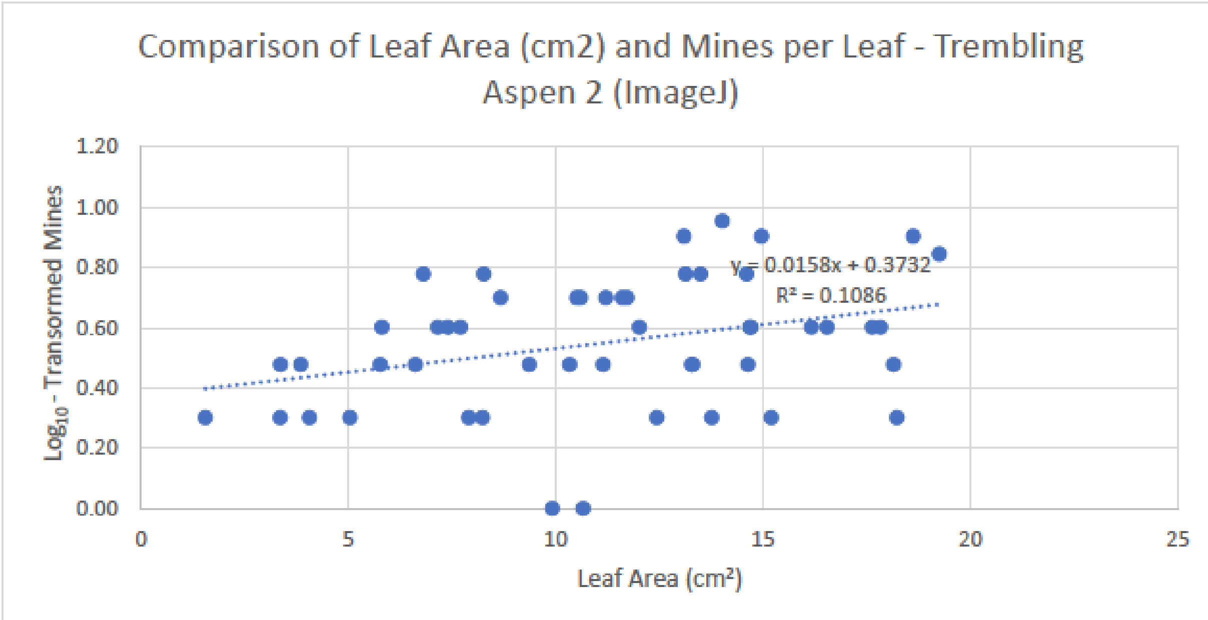


Figure 29 - scatter plot displaying leaf area (ImageJ) and the number of mines per leaf for trembling aspen 2, number of mines per leaf has been log transformed

Table 7 - Regression statistics: trembling aspen leaf blotch mines per leaf and leaf surface area (cm2) - ImageJ

Regression Statistics	
Multiple R	0.329499076
R Square	0.108569641
Adjusted R Square	0.089998175
Standard Error	0.208063347
Observations	50

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.25307741	0.253077	5.846046	0.019457419
Residual	48	2.077937107	0.04329		
Total	49	2.331014517			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.373160697	0.078640029	4.745175	1.91E-05	0.215044322	0.531277072	0.215044322	0.531277072
X Variable 1	0.01579994	0.00653468	2.41786	0.019457	0.002661085	0.028938795	0.002661085	0.028938795

A t-test performed comparing SketchAndCalc and ImageJ for the purpose of leaf area measurement yielded no significant difference between the two methods for this application (H_0: no significant difference between either method of leaf area calculation for balsam poplar, $t_{0.025}$ df = 49, t = -0.8, p = 0.45, FTR H_0; H_0: no significant difference between either method of leaf area calculation for trembling aspen, $t_{0.025}$ df = 49, t = -0.98, p = 0.33, FTR H_0).

Table 8 - t-test of average leaf surface area (cm2) found for both tree species using SketchAndCalc and ImageJ

Null Hypothesis: There is no difference between either method in measuring surface area for either tree species

Balsam Poplar

t-Test: Paired Two Sample for Means

	ImageJ	*SketchAndCalc*
Mean	24.25798	25.4284
Variance	67.35444639	74.18145861
Observations	50	50
Pearson Correlation	0.153213126	
Hypothesized Mean D	0	
df	49	
t Stat	-0.755893545	
P(T<=t) one-tail	0.226665853	
t Critical one-tail	1.676550893	
P(T<=t) two-tail	0.453331706	
t Critical two-tail	2.009575237	

P = 0.453 > 0.025

t<tCrit = FTR

No significant difference

Trembling Aspen

t-Test: Paired Two Sample for Means

	ImageJ	*SketchAndCalc*
Mean	11.1601	12.049
Variance	20.68932964	23.76752347
Observations	50	50
Pearson Correlation	0.075935799	
Hypothesized Mean	0	
df	49	
t Stat	-0.98056109	
P(T<=t) one-tail	0.165813023	
t Critical one-tail	1.676550893	
P(T<=t) two-tail	0.331626046	
t Critical two-tail	2.009575237	

P = 0.332 > 0.025

t<tCrit = FTR

No significant difference

DISCUSSION

The distribution of leaf blotch mine occurrences on sample leaves was found to correspond to a poisson-type pattern (Figures 19 - 26). This result is in contrast to studies conducted by Auerbach (1989) and Simberloff (1987) who found that leaf mines created by *Lithocolletis quercus* and *Stilbosis quadricustatella* did not follow a poisson type pattern – However, Auberbach and Simberloff studied linear leaf mines and not leaf blotch mines, which may account for the differences between studies. Regression analyses for both trembling aspen and balsam poplar suggest a positive linear relationship between leaf area and the number of leaf blotch mines on each leaf, with a greater leaf area relating to a higher number of mines. These findings are supported by several other studies in which positive linear relationships were also found between leaf area and the number of leaf blotch mines present (Auerbach 1989, Simberloff 1987, Tuomi 1981). The relationship between leaf area and number of leaf blotch mines suggests that adult female *P. nipigon* possibly select leaves with more tissue available for larvae to feed on. If several eggs were to be deposited on a smaller leaf, it may be more likely that these larvae might not survive or have reduced survival due to intraspecific competition.

Comparisons of trembling aspen and balsam poplar leaf areas showed that balsam poplar has , on average, a much larger leaf area than trembling aspen. It is interesting to note that, while a significant difference in leaf size was found between the two tree species, and a positive linear relationship between larger leaf area and more leaf blotch mines was found, the average number of leaf blotch mines on either tree was not found to be significantly different. This could suggest that female *P. nipigon* moths tend to oviposit on host leaves in an opportunistic manner. If a female lands on a trembling aspen, she may simply select larger leaves to lay eggs on, as opposed to trying to find a balsam poplar host tree, given that they have larger leaves on average. Alternatively, the poisson-distributed pattern of leaf blotch mines suggests that female moths select leaves at random, with some leaves ending up with numerous mines, while a few leaves escape attack completely.

A comparison of the two methods of measuring leaf area found that both SketchAndCalc and ImageJ were appropriate tools for measuring balsam poplar and trembling aspen leaf areas, as there was no significant difference found between the leaf area data provided by the two methods. SketchAndCalc did find, on average, larger leaf areas. This was likely due to the potential for measurement error when tracing leaf margins, as it would take meticulous care and effort to trace each serration on the leaf margin. ImageJ mitigates this issue through balancing the image thresholds and automatically highlighting the borders of the leaf – including each serration on the leaf margin. While the issue of an exaggerated border through leaf area measurement when using SketchAndCalc does exist, it was not found to be of significance for the purpose of this study. It is important to note, however, that both of these methods assume that any damage to the leaf margin occurred prior to oviposition on the leaves.

Other issues discovered that may skew leaf areas was the potential folding or curling of leaves. This issue was mitigated when scanning leaves for ImageJ, as the lid of the scanner flattened leaf surfaces, eliminating any folds present. When photographing leaves for SketchAndCalc it would have been beneficial to press the leaves between the lightboard and a transparent surface, such as a glass plate, effectively removing any folds in the leaf. Perhaps the optimal method of measuring leaf area would be through the use of a Li-Cor leaf-area meter. This tool uses a two-belt system, feeding leaves between the transparent belts using a roller which flattens them, eliminating any curls present. This tool is accurate to 0.1 mm^2 and has been used to measure leaf area in similar studies (Auerbach 1989).

CONCLUSION

The two tools used for measuring leaves in this study are both viable means of estimating leaf surface area to a high degree of accuracy. While SketchAndCalc was found to have higher estimates of leaf area, it was not significant enough to have a major impact on findings. Mine frequency distributions across all sample trees followed a poisson-distributed pattern and a positive linear relationship between leaf surface area and number of leaf blotch mines present were found, with larger leaves more likely to have a higher leaf blotch mine count. There was not a significantly larger presence of mines on the tree species with larger leaves (balsam poplar) – suggesting that oviposition by adult female *P. nipigon* moths occurs in an opportunistic manner. Future studies could examine potential effects of *P. nipigon* outbreaks on balsam poplar and trembling aspen growth, as evidenced by reductions in radial growth due to a loss of photosynthetic area.

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APPENDICES

APPENDIX A

Leaf blotch mine frequency, distribution, and leaf surface area data

Table 9 - Leaf mine counts across all sample trees

	Mines Counted Per Leaf				
Leaf Sample No	Balsam Poplar 1	Balsam Poplar 2	Balsam Poplar 3	Trembling Aspen 1	Trembling Aspen 2
1	5	0	1	4	2
2	6	2	3	4	2
3	4	3	3	2	5
4	12	3	0	4	6
5	3	2	0	2	2
6	3	4	6	5	3
7	5	2	8	6	2
8	4	4	5	5	1
9	1	4	7	5	6
10	2	2	8	3	0
11	3	5	2	5	7
12	1	8	1	1	1
13	5	8	7	2	2
14	1	0	5	7	3
15	4	5	4	5	4
16	5	8	3	4	4
17	5	4	2	5	3
18	6	4	4	2	3
19	4	6	4	2	2
20	9	1	8	5	4
21	4	2	6	5	3
22	4	4	6	2	7
23	5	6	3	1	5
24	2	6	11	6	6
25	6	3	8	8	4
26	5	3	10	5	4
27	3	2	7	2	2
28	5	14	5	7	2
29	5	9	7	5	3
30	3	4	6	6	0
31	4	3	8	5	2
32	9	3	9	3	2
33	6	2	8	5	1
34	8	4	1	3	3
35	4	3	8	5	4
36	14	4	0	5	6
37	8	2	3	5	3
38	4	2	3	6	3
39	12	3	3	6	6
40	8	7	2	6	3
41	1	3	0	4	3
42	9	3	3	6	3
43	1	4	3	4	6
44	7	2	1	7	1
45	3	2	5	5	8
46	5	2	2	3	4
47	0	1	6	5	3
48	8	5	4	3	3
49	8	7	5	4	2
50	7	3	2	5	0
51	7	3	5	4	4
52	6	3	7	3	4
53	4	3	2	5	2
54	5	6	7	6	4
55	5	4	7	3	2
56	7	3	4	5	7
57	3	5	5	2	2
58	5	4	0	2	5
59	9	6	7	4	3
60	3	5	5	8	3
61	5	4	4	6	4
62	3	2	8	2	3
63	7	3	4	3	2
64	5	3	4	2	1
65	7	5	4	3	3
66	8	4	2	6	5
67	3	3	2	8	7
68	5	1	4	2	10
69	9	4	3	5	1
70	1	3	6	4	1
71	7	4	5	3	4
72	4	4	3	5	2
73	3	2	5	1	5
74	5	3	2	4	5
75	9	1	3	4	4
76	7	2	2	3	7
77	6	5	2	3	4
78	3	1	6	5	0
79	5	11	6	4	7
80	5	6	2	6	2
81	2	10	8	5	7
82	1	9	5	4	6
83	4	3	3	4	10
84	3	0	8	4	6
85	0	3	9	6	6
86	3	5	6	2	1
87	9	8	3	4	5
88	4	2	2	4	8
89	4	7	9	4	5
90	2	5	0	4	2
91	8	7	3	5	2
92	6	3	4	4	1
93	7	3	5	6	5
94	3	4	4	3	2
95	4	2	3	5	5
96	5	3	0	4	4
97	2	1	3	3	3
98	3	4	4	1	3
99	3	4	4	2	4
100	1	2	5	3	5
Total	491	391	440	418	367
Average	4.91	3.91	4.4	4.18	3.67
Species Average		4.41		3.93	
Total Average			4.214		

Table 10 - Log transformed data, leaf blotch mine counts across all sample trees

	Log(x+1)				
	Mines Couned Per Leaf				
Leaf Sample No	Balsam Poplar 1	Balsam Poplar 2	Balsam Poplar 3	Trembling Aspen 1	Trembling Aspen 2
1	0.778	0.000	0.301	0.699	0.477
2	0.845	0.477	0.602	0.699	0.477
3	0.699	0.602	0.602	0.477	0.778
4	1.114	0.602	0.000	0.699	0.845
5	0.602	0.477	0.000	0.477	0.477
6	0.602	0.699	0.845	0.778	0.602
7	0.778	0.477	0.954	0.845	0.477
8	0.699	0.699	0.778	0.778	0.301
9	0.301	0.699	0.903	0.778	0.845
10	0.477	0.477	0.954	0.602	0.000
11	0.602	0.778	0.477	0.778	0.903
12	0.301	0.954	0.301	0.301	0.301
13	0.778	0.954	0.903	0.477	0.477
14	0.301	0.000	0.778	0.903	0.602
15	0.699	0.778	0.699	0.778	0.699
16	0.778	0.954	0.602	0.699	0.699
17	0.778	0.699	0.477	0.778	0.602
18	0.845	0.699	0.699	0.477	0.602
19	0.699	0.845	0.699	0.477	0.477
20	1.000	0.301	0.954	0.778	0.699
21	0.699	0.477	0.845	0.778	0.602
22	0.699	0.699	0.845	0.477	0.903
23	0.778	0.845	0.602	0.301	0.778
24	0.477	0.845	1.079	0.845	0.845
25	0.845	0.602	0.954	0.954	0.699
26	0.778	0.602	1.041	0.778	0.699
27	0.602	0.477	0.903	0.477	0.477
28	0.778	1.176	0.778	0.903	0.477
29	0.778	1.000	0.903	0.778	0.602
30	0.602	0.699	0.845	0.845	0.000
31	0.699	0.602	0.954	0.778	0.477
32	1.000	0.602	1.000	0.602	0.477
33	0.845	0.477	0.954	0.778	0.301
34	0.954	0.699	0.301	0.602	0.602
35	0.699	0.602	0.954	0.778	0.699
36	1.176	0.699	0.000	0.778	0.845
37	0.954	0.477	0.602	0.778	0.602
38	0.699	0.477	0.602	0.845	0.602
39	1.114	0.602	0.602	0.845	0.845
40	0.954	0.903	0.477	0.845	0.602
41	0.301	0.602	0.000	0.699	0.602
42	1.000	0.602	0.602	0.845	0.602
43	0.301	0.699	0.602	0.699	0.845
44	0.903	0.477	0.301	0.903	0.301
45	0.602	0.477	0.778	0.778	0.954
46	0.778	0.477	0.477	0.602	0.699
47	0.000	0.301	0.845	0.778	0.602
48	0.954	0.778	0.699	0.602	0.602
49	0.954	0.903	0.778	0.699	0.477
50	0.903	0.602	0.477	0.778	0.000
51	0.903	0.602	0.778	0.699	0.699
52	0.845	0.602	0.903	0.602	0.699
53	0.699	0.602	0.477	0.778	0.477
54	0.778	0.845	0.903	0.845	0.699
55	0.778	0.699	0.903	0.602	0.477
56	0.903	0.602	0.699	0.778	0.903
57	0.602	0.778	0.778	0.477	0.477
58	0.778	0.699	0.000	0.477	0.778
59	1.000	0.845	0.903	0.699	0.602
60	0.602	0.778	0.778	0.954	0.602
61	0.778	0.699	0.699	0.845	0.699
62	0.602	0.477	0.954	0.477	0.602
63	0.903	0.602	0.699	0.602	0.477
64	0.778	0.602	0.699	0.477	0.301
65	0.903	0.778	0.699	0.602	0.602
66	0.954	0.699	0.477	0.845	0.778
67	0.602	0.602	0.477	0.954	0.903
68	0.778	0.301	0.699	0.477	1.041
69	1.000	0.699	0.602	0.778	0.301
70	0.301	0.602	0.845	0.699	0.301
71	0.903	0.699	0.778	0.602	0.699
72	0.699	0.699	0.602	0.778	0.477
73	0.602	0.477	0.778	0.301	0.778
74	0.778	0.602	0.477	0.699	0.778
75	1.000	0.301	0.602	0.699	0.699
76	0.903	0.477	0.477	0.602	0.903
77	0.845	0.778	0.477	0.602	0.699
78	0.602	0.301	0.845	0.778	0.000
79	0.778	1.079	0.845	0.699	0.903
80	0.778	0.845	0.477	0.845	0.477
81	0.477	1.041	0.954	0.778	0.903
82	0.301	1.000	0.778	0.699	0.845
83	0.699	0.602	0.602	0.699	1.041
84	0.602	0.000	0.954	0.699	0.845
85	0.000	0.602	1.000	0.845	0.845
86	0.602	0.778	0.845	0.477	0.301
87	1.000	0.954	0.602	0.699	0.778
88	0.699	0.477	0.477	0.699	0.954
89	0.699	0.903	1.000	0.699	0.778
90	0.477	0.778	0.000	0.699	0.477
91	0.954	0.903	0.602	0.778	0.477
92	0.845	0.602	0.699	0.699	0.301
93	0.903	0.602	0.778	0.845	0.778
94	0.602	0.699	0.699	0.602	0.477
95	0.699	0.477	0.602	0.778	0.778
96	0.778	0.602	0.000	0.699	0.699
97	0.477	0.301	0.602	0.602	0.602
98	0.602	0.699	0.699	0.301	0.602
99	0.602	0.699	0.699	0.477	0.699
100	0.301	0.477	0.778	0.602	0.778
Total	72.204	64.225	67.037	69.082	61.955
Average	0.722	0.642	0.670	0.691	0.620
Species Average		0.68			0.66

Table 11 - Leaf blotch mine frequency distributions, total or both species and across each sample tree

Pt	
Mine Count	Frequency
0	4
1	13
2	34
3	35
4	38
5	37
6	22
7	10
8	5
9	0
10	2
11	0
12	0
13	0
14	0

Bp	
Mine Count	Frequency
0	12
1	18
2	36
3	59
4	49
5	42
6	22
7	21
8	21
9	12
10	2
11	2
12	2
13	0
14	2

Bp1	
Mine Count	Frequency
0	2
1	8
2	5
3	17
4	15
5	20
6	7
7	9
8	7
9	7
10	0
11	0
12	2
13	0
14	1

Bp2	
Mine Count	Frequency
0	3
1	6
2	18
3	25
4	20
5	9
6	6
7	4
8	4
9	2
10	1
11	1
12	0
13	0
14	1

Bp3	
Mine Count	Frequency
0	7
1	4
2	13
3	17
4	14
5	13
6	9
7	8
8	10
9	3
10	1
11	1
12	0
13	0
14	0

Pt1	
Mine Count	Frequency
0	0
1	4
2	14
3	15
4	22
5	26
6	13
7	3
8	3
9	0
10	0
11	0
12	0
13	0
14	0

Pt2	
Mine Count	Frequency
0	4
1	9
2	20
3	20
4	16
5	11
6	9
7	7
8	2
9	0
10	2
11	0
12	0
13	0
14	0

Table 12 - Leaf surface area results using SketchAndCalc method for two sample trees

Balsam Poplar 3			
Leaf Sample No	Surface Area (cm2)	Mines	Mines - Log(x+1)
1	26.97	4	0.70
2	18	0	0.00
3	32.29	2	0.48
4	23.3	2	0.48
5	28.35	3	0.60
6	16.57	1	0.30
7	25.76	3	0.60
8	21.42	2	0.48
9	24.35	3	0.60
10	22.62	3	0.60
11	12.16	0	0.00
12	23.01	5	0.78
13	19.55	1	0.30
14	21.09	6	0.85
15	11.25	0	0.00
16	27.02	1	0.30
17	24.69	4	0.70
18	20.33	6	0.85
19	26.43	4	0.70
20	26.32	3	0.60
21	26.11	5	0.78
22	11.05	2	0.48
23	34.34	4	0.70
24	20.61	2	0.48
25	40.01	3	0.60
26	42.40	9	1.00
27	20.67	3	0.60
28	35.23	5	0.78
29	21.85	4	0.70
30	15.2	2	0.48
31	15.54	1	0.30
32	19.62	4	0.70
33	21.65	3	0.60
34	16.44	6	0.85
35	42.86	8	0.95
36	16.71	3	0.60
37	32.73	6	0.85
38	41.1	5	0.78
39	25.16	5	0.78
40	12.14	2	0.48
41	42.57	4	0.70
42	37.69	6	0.85
43	20.45	1	0.30
44	30.34	6	0.85
45	40.47	6	0.85
46	23.04	5	0.78
47	33.8	5	0.78
48	26.76	2	0.48
49	23.74	5	0.78
50	29.66	6	0.85
Average	25.43	3.62	

Trembling Aspen 2			
Leaf Sample No	Surface Area (cm2)	Mines	Mines - Log(x+1)
1	16.39	2	0.48
2	16.44	3	0.60
3	11.48	2	0.48
4	11.64	0	0.00
5	16.89	1	0.30
6	19.05	3	0.60
7	13.73	5	0.78
8	19.61	3	0.60
9	15.65	1	0.30
10	13.25	2	0.48
11	14.46	2	0.48
12	19.55	2	0.48
13	10.99	4	0.70
14	8.51	4	0.70
15	9.32	0	0.00
16	7.8	3	0.60
17	17.72	3	0.60
18	17.49	3	0.60
19	15.82	5	0.78
20	15.3	8	0.95
21	13.63	3	0.60
22	15.54	2	0.48
23	8.52	1	0.30
24	11.17	0	0.00
25	8.42	3	0.60
26	4.22	1	0.30
27	19.5	3	0.60
28	10.03	5	0.78
29	3.84	2	0.48
30	5.47	1	0.30
31	13.85	4	0.70
32	11.18	4	0.70
33	14.37	5	0.78
34	14.19	7	0.90
35	5.6	3	0.60
36	20.25	7	0.90
37	12.12	4	0.70
38	7.96	4	0.70
39	3.95	1	0.30
40	3.88	1	0.30
41	8.4	3	0.60
42	1.66	1	0.30
43	7.21	2	0.48
44	10.94	2	0.48
45	11.99	1	0.30
46	13.82	8	0.95
47	19.84	7	0.90
48	11.48	2	0.48
49	5.99	2	0.48
50	12.34	4	0.70
Average	12.05	2.98	

Table 13 - Leaf surface area results using ImageJ method for two sample trees

Balsam Poplar 3			
Leaf Sample No	Surface Area (cm2)	Mines	Mines - Log(x+1)
1	26.45	1	0.30
2	35.75	6	0.85
3	14.55	1	0.30
4	26.95	3	0.60
5	26.01	5	0.78
6	9.84	0	0.00
7	10.46	2	0.48
8	34.18	4	0.70
9	42.24	5	0.78
10	20.09	2	0.48
11	17.90	2	0.48
12	15.15	1	0.30
13	26.92	6	0.85
14	21.01	3	0.60
15	14.09	2	0.48
16	22.13	5	0.78
17	23.02	4	0.70
18	20.30	4	0.70
19	23.02	3	0.60
20	17.41	1	0.30
21	25.37	6	0.85
22	27.43	2	0.48
23	23.82	2	0.48
24	26.41	2	0.48
25	30.25	5	0.78
26	39.34	9	1.00
27	25.57	3	0.60
28	24.56	3	0.60
29	17.10	1	0.30
30	15.65	0	0.00
31	11.32	0	0.00
32	21.20	3	0.60
33	31.58	4	0.70
34	26.74	6	0.85
35	30.24	6	0.85
36	26.49	4	0.70
37	10.42	2	0.48
38	22.49	5	0.78
39	40.36	4	0.70
40	39.18	4	0.70
41	22.65	4	0.70
42	20.75	4	0.70
43	36.31	9	1.00
44	34.48	6	0.85
45	32.52	5	0.78
46	16.44	3	0.60
47	30.81	6	0.85
48	15.61	6	0.85
49	19.29	3	0.60
50	21.11	4	0.70
Average	24.26	3.62	

Trembling Aspen 2			
Leaf Sample No	Surface Area (cm2)	Mines	Mines - Log(x+1)
1	14.60	5	0.78
2	8.26	5	0.78
3	13.76	1	0.30
4	10.33	2	0.48
5	5.04	1	0.30
6	18.21	1	0.30
7	17.62	3	0.60
8	14.63	2	0.48
9	7.90	1	0.30
10	17.82	3	0.60
11	13.12	5	0.78
12	13.30	2	0.48
13	9.92	0	0.00
14	10.65	0	0.00
15	4.05	1	0.30
16	8.66	4	0.70
17	11.20	4	0.70
18	7.70	3	0.60
19	5.80	3	0.60
20	6.61	2	0.48
21	12.43	1	0.30
22	13.27	2	0.48
23	1.55	1	0.30
24	14.95	7	0.90
25	14.70	3	0.60
26	16.53	3	0.60
27	14.01	8	0.95
28	16.16	3	0.60
29	15.19	1	0.30
30	11.60	4	0.70
31	13.48	5	0.78
32	11.14	2	0.48
33	18.14	2	0.48
34	3.36	2	0.48
35	7.40	3	0.60
36	14.67	3	0.60
37	7.15	3	0.60
38	9.36	2	0.48
39	8.23	1	0.30
40	11.71	4	0.70
41	5.76	2	0.48
42	12.01	3	0.60
43	18.61	7	0.90
44	13.08	7	0.90
45	3.35	1	0.30
46	3.85	2	0.48
47	10.59	4	0.70
48	10.50	4	0.70
49	6.80	5	0.78
50	19.24	6	0.85
Average	11.16	2.98	

Appendix B

Catalogue of sample leaves for surface area analysis – ImageJ – balsam poplar

Leaf 1

Leaf 2

Leaf 3

Leaf 4

Leaf 5

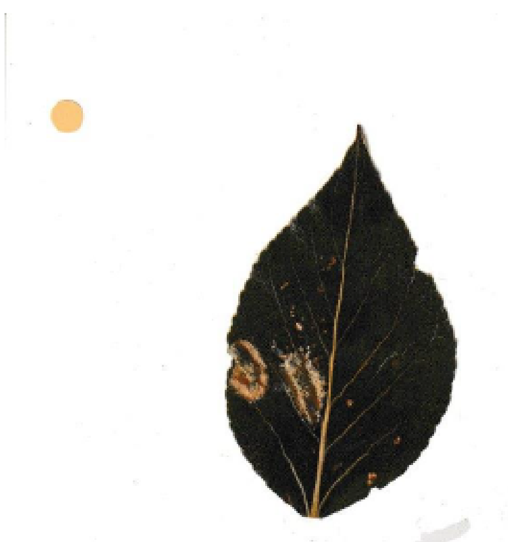
Leaf 6

Leaf 7

Leaf 8

Leaf 9

Leaf 10



Leaf 11

Leaf 12

Leaf 13

Leaf 14

Leaf 15

Leaf 16

Leaf 17

Leaf 18

Leaf 19

Leaf 20

Leaf 21

Leaf 22

Leaf 23

Leaf 24

Leaf 25

Leaf 26

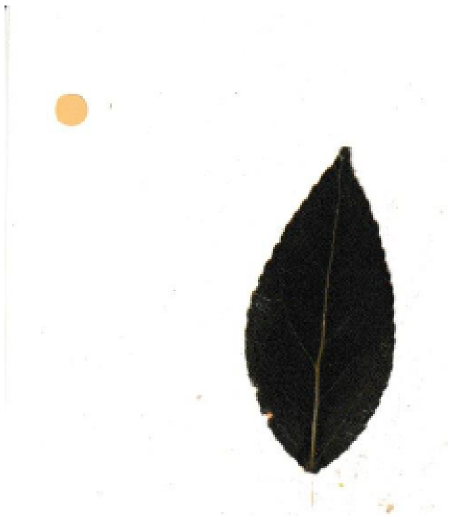
Leaf 27

Leaf 28

Leaf 29

Leaf 30

Leaf 31



Leaf 32

Leaf 33

Leaf 34

Leaf 34

Leaf 36

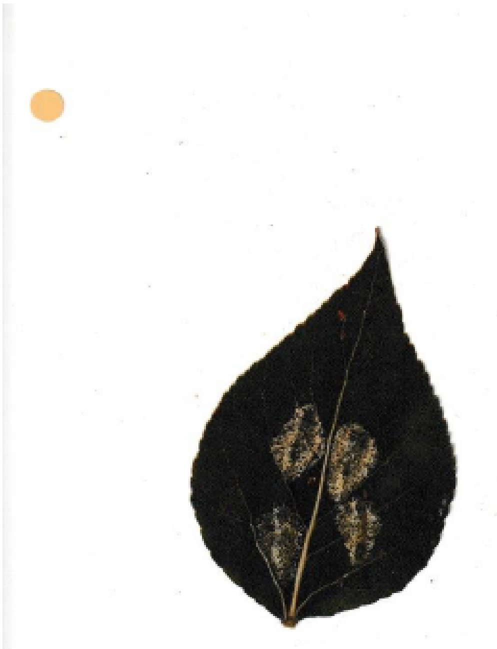
Leaf 37

Leaf 38

Leaf 39

Leaf 40

Leaf 41



Leaf 42

Leaf 43

Leaf 44

Leaf 45

Leaf 46

Leaf 47

Leaf 48

Leaf 49

Leaf 50

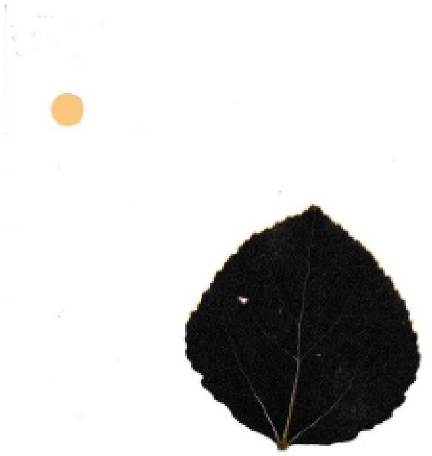
APPENDIX C

Catalogue of sample leaves for surface area analysis – ImageJ – trembling aspen

Leaf 1

Leaf 2

Leaf 3



Leaf 4

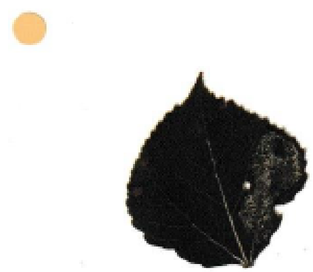
Leaf 5

Leaf 6

Leaf 7

Leaf 8

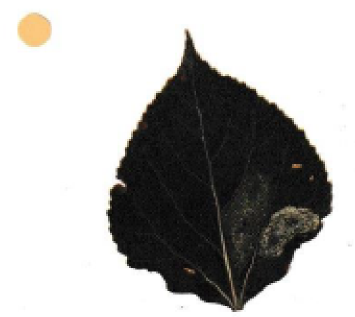
Leaf 9



Leaf 10

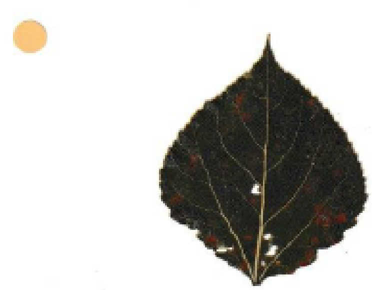
Leaf 11

Leaf 12



Leaf 13

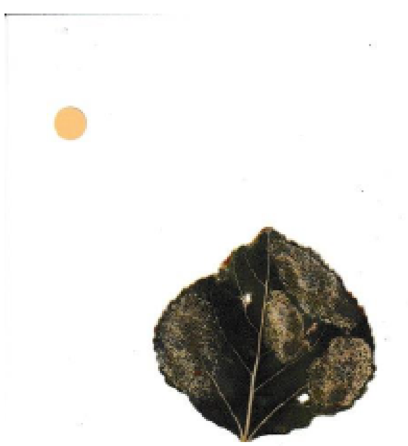
Leaf 14



Leaf 15

Leaf 16

Leaf 17



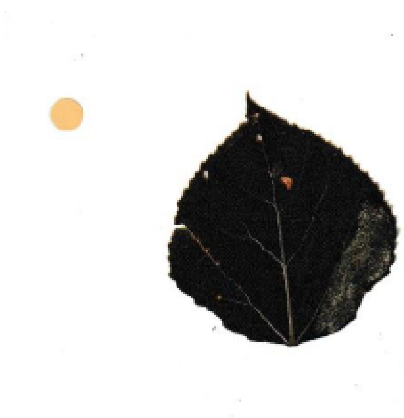
Leaf 18

Leaf 19

Leaf 20

Leaf 21

Leaf 22



Leaf 23

Leaf 24

Leaf 25

Leaf 26

Leaf 27

Leaf 28

Leaf 29

Leaf 30

Leaf 31

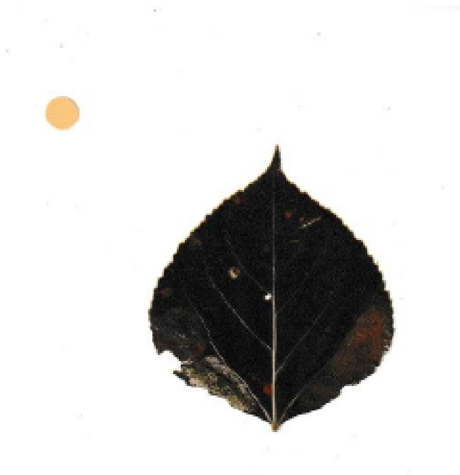
Leaf 32

Leaf 33

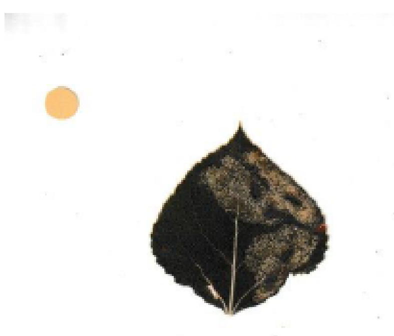
Leaf 34

Leaf 35

Leaf 36

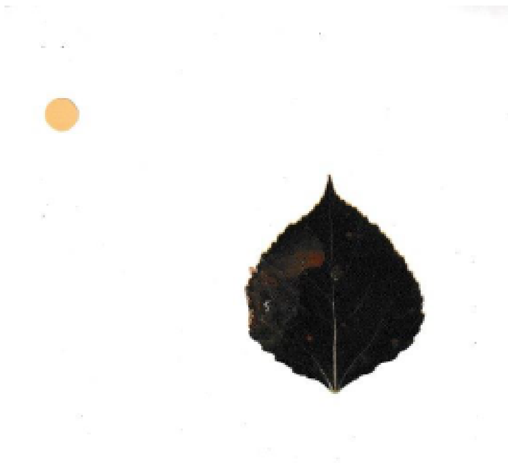


Leaf 37



Leaf 38

Leaf 39



Leaf 40

Leaf 41

Leaf 42

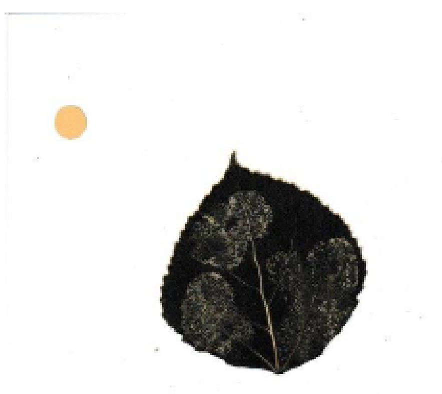
Leaf 43

Leaf 44

Leaf 45

Leaf 46

Leaf 47



Leaf 48

Leaf 49

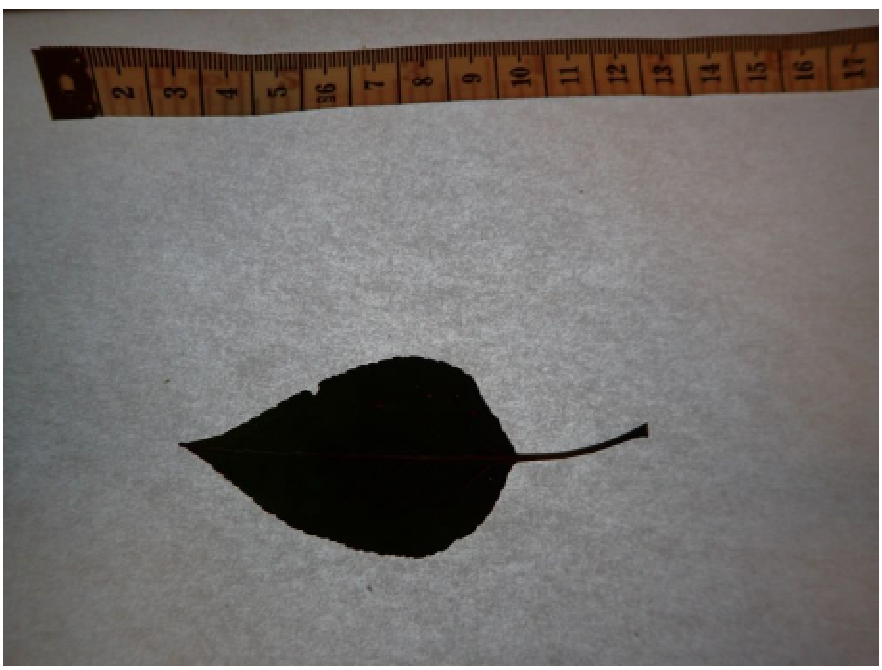
Leaf 50

APPENDIX D

Catalogue of sample leaves for surface area analysis – SketchAndCalc – balsam poplar

Leaf 1

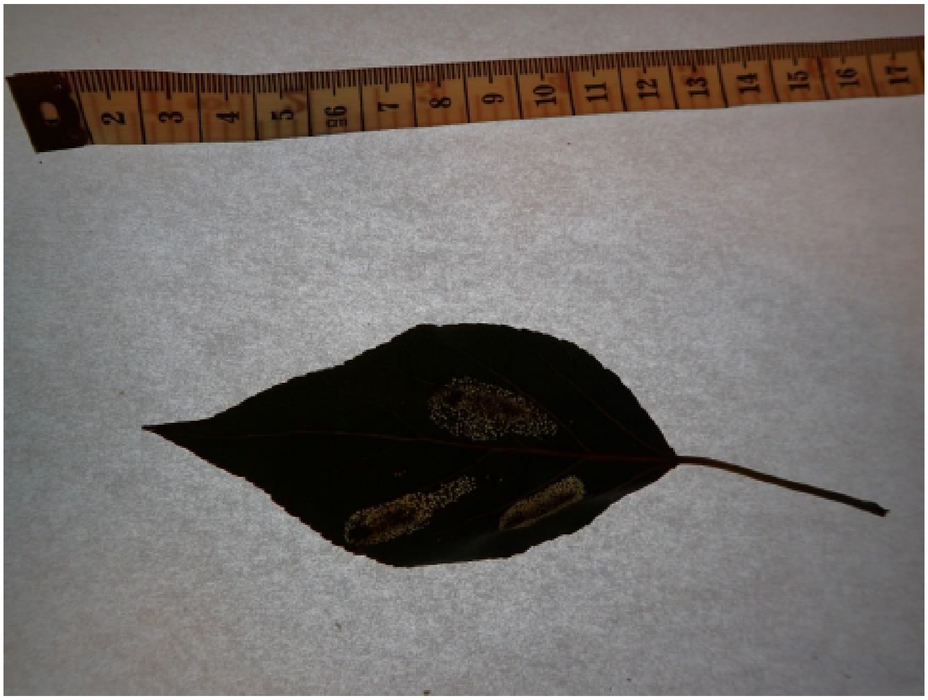
Leaf 2



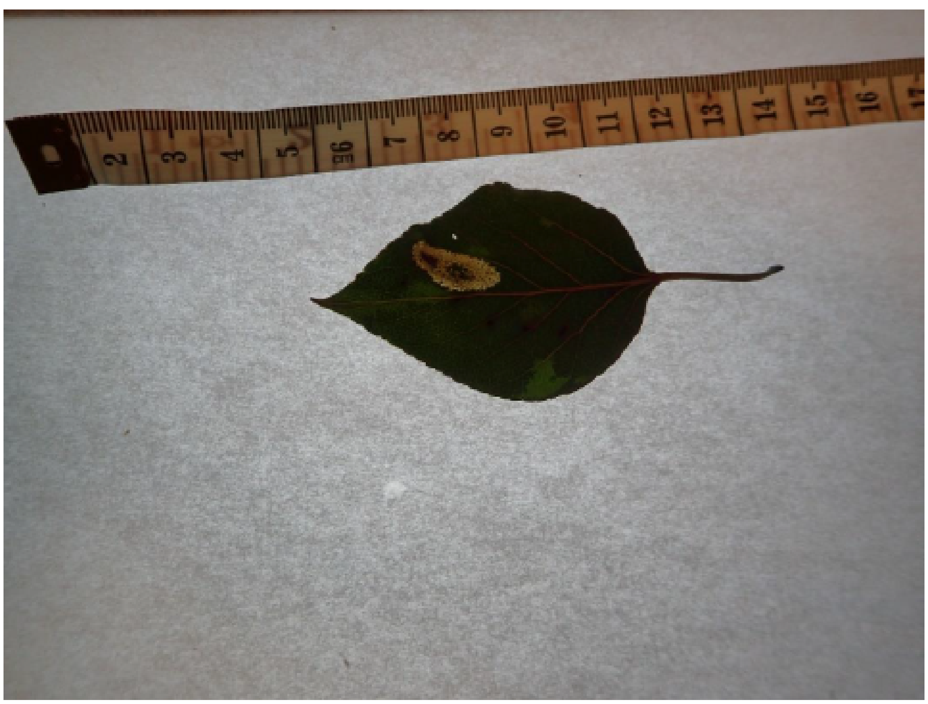
Leaf 3

Leaf 4

Leaf 5



Leaf 6



Leaf 7

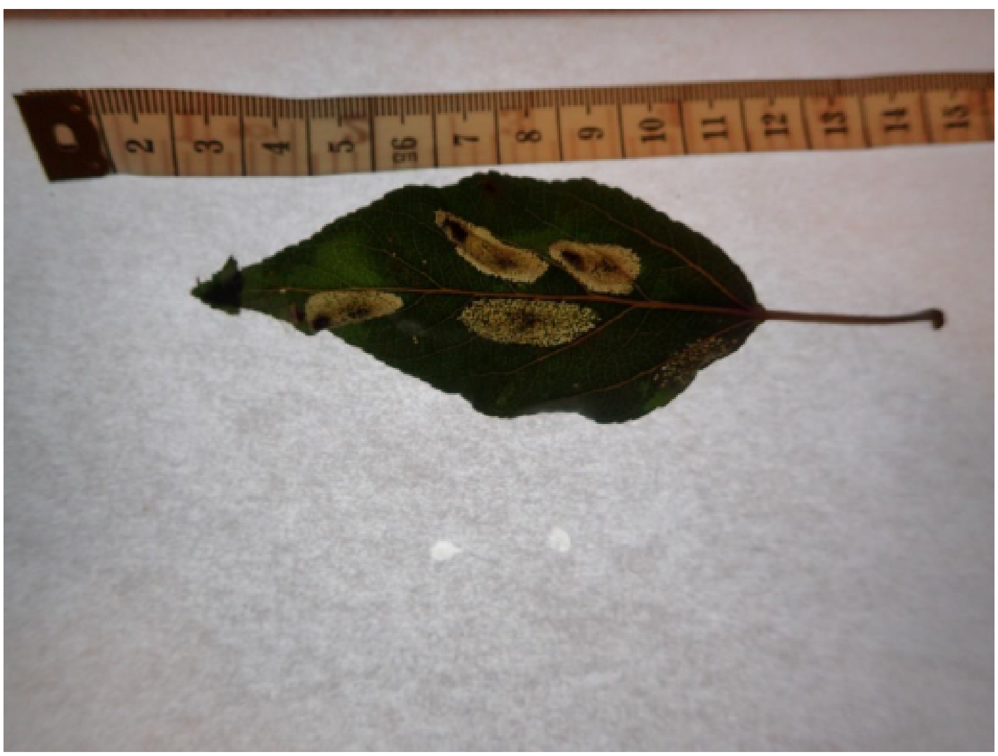
Leaf 8

Leaf 9

Leaf 10

Leaf 11

Leaf 12



Leaf 13

Leaf 14

Leaf 15

Leaf 16

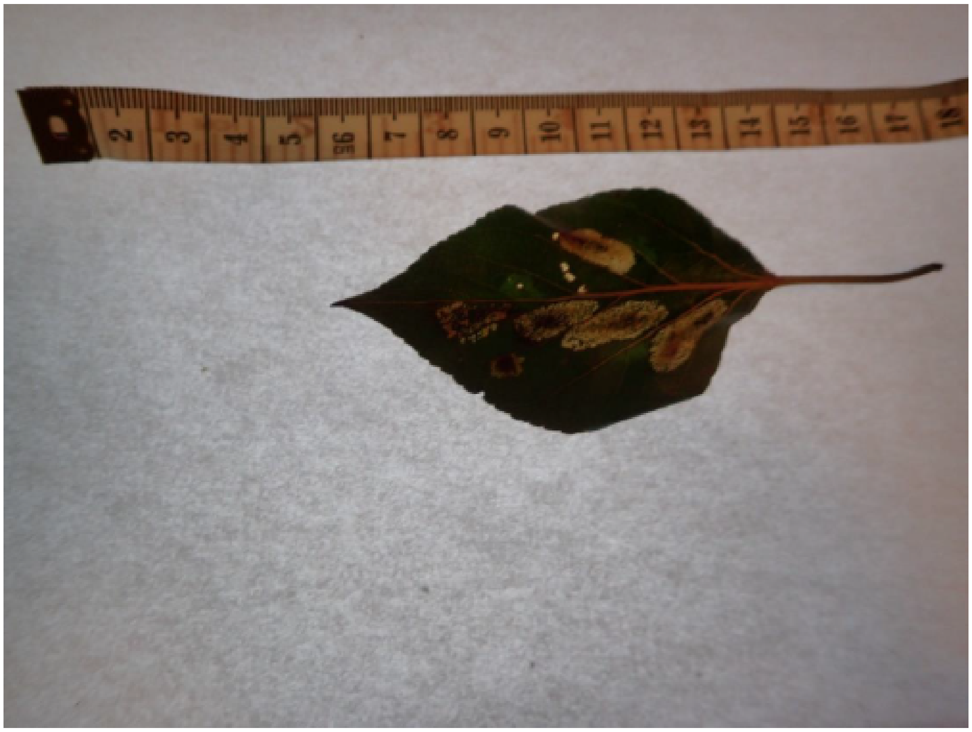
Leaf 17

Leaf 18

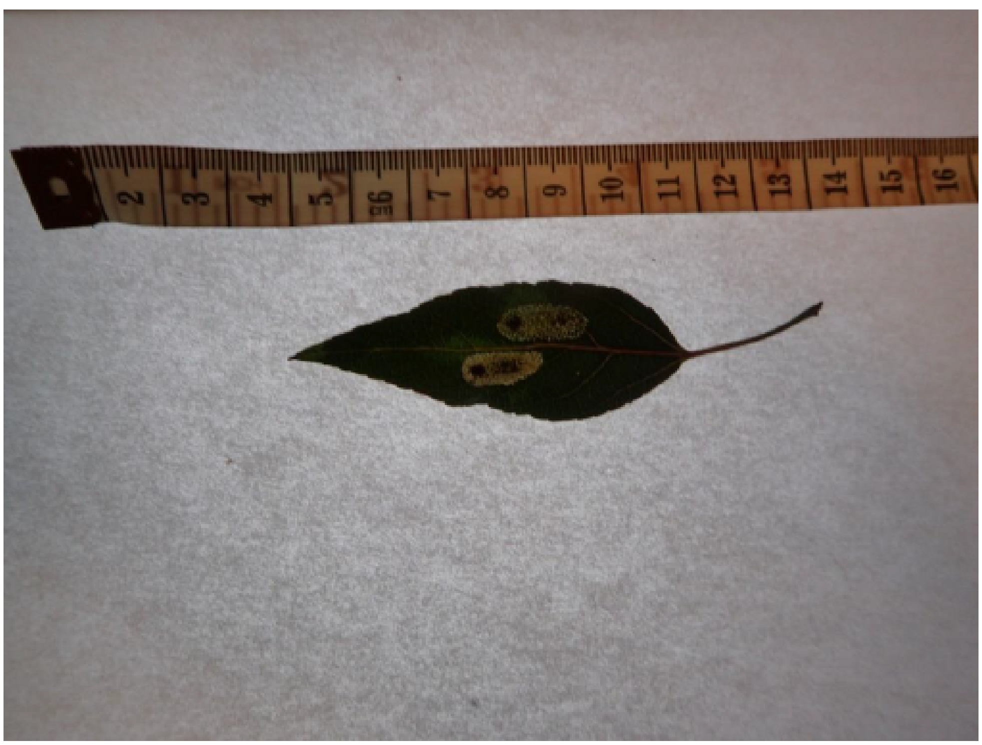
Leaf 19

Leaf 20

Leaf 21



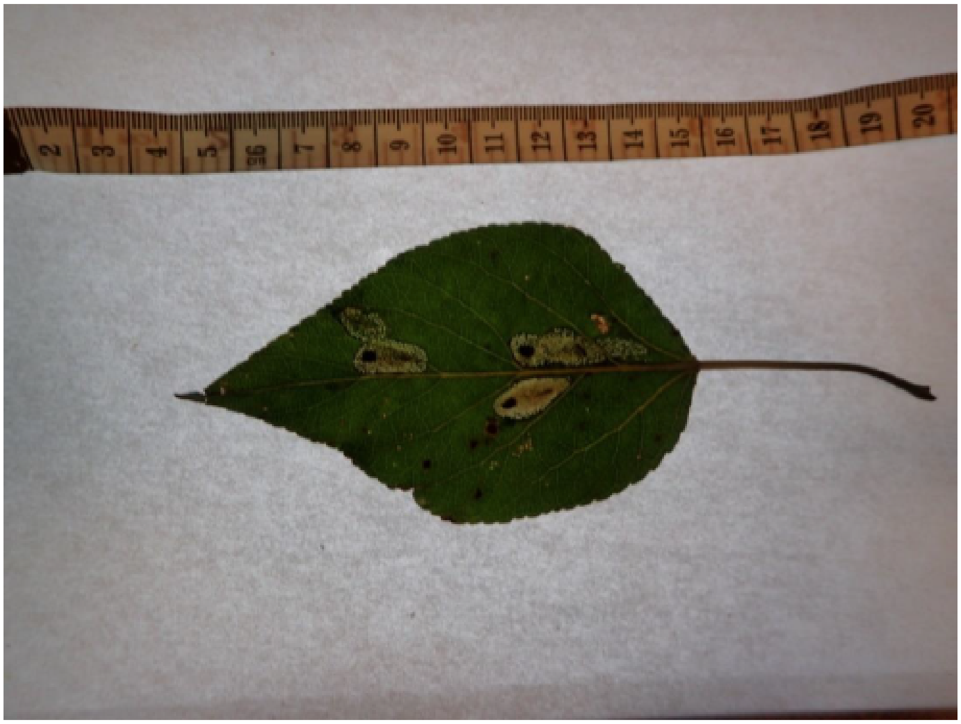
Leaf 22



Leaf 23

Leaf 24

Leaf 25

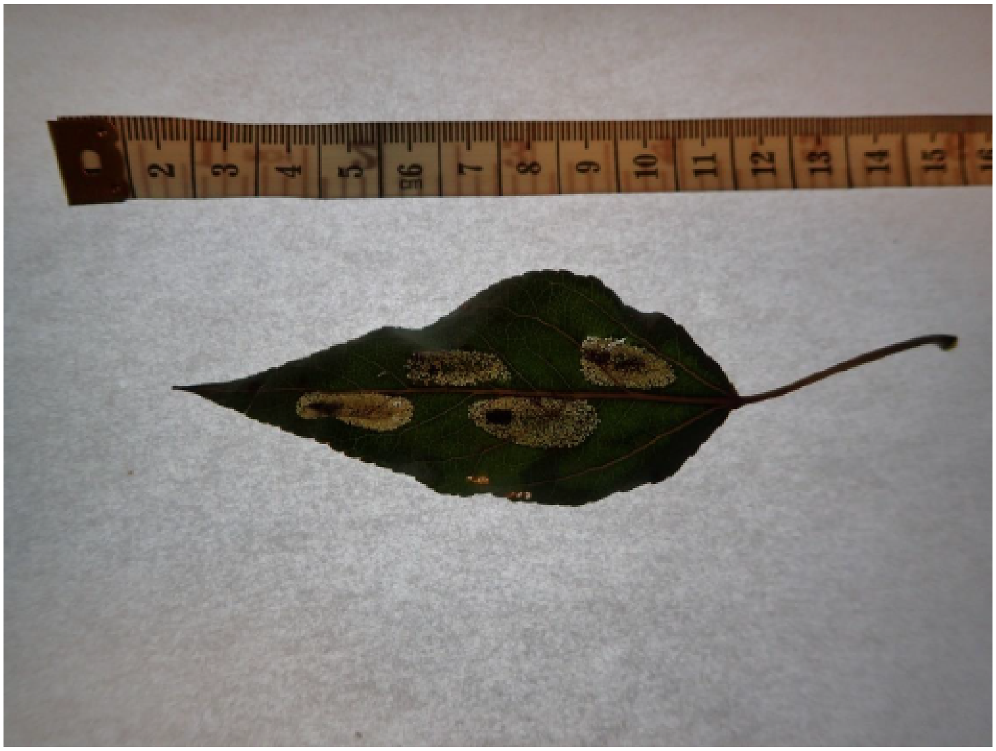


Leaf 26

Leaf 27

Leaf 28

Leaf 29



Leaf 30

Leaf 31

Leaf 32

Leaf 33

Leaf 34

Leaf 35

Leaf 36

Leaf 37

Leaf 38

Leaf 39

Leaf 40

Leaf 41

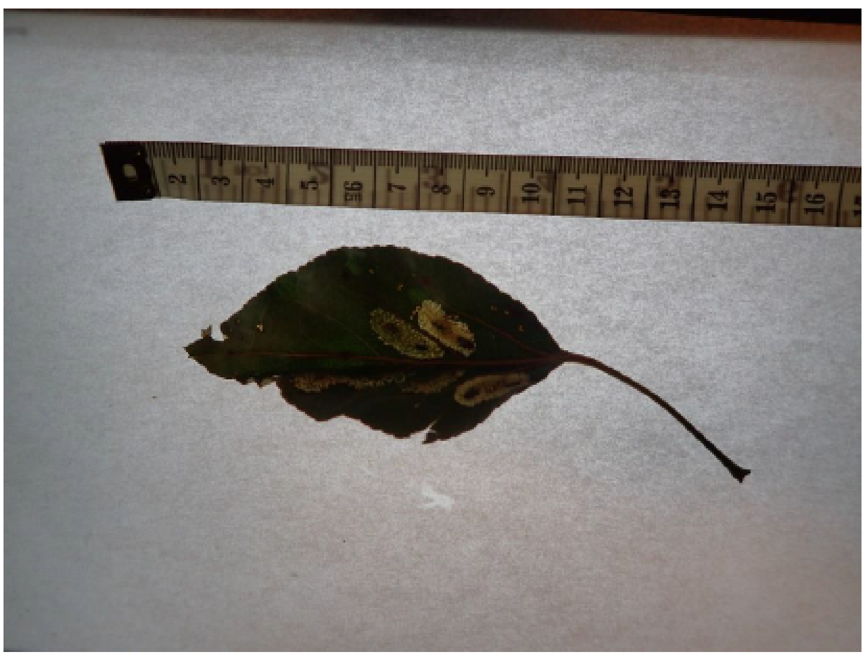
Leaf 42

Leaf 43

Leaf 44

Leaf 45

Leaf 46



Leaf 47

Leaf 48

Leaf 49

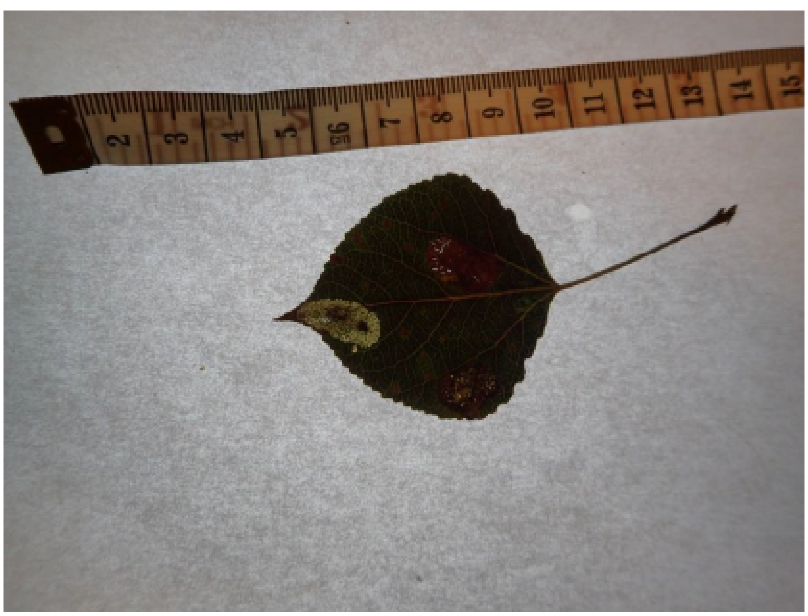
Leaf 50

APPENDIX E

Catalogue of sample leaves for surface area analysis – SketchAndCalc – trembling aspen

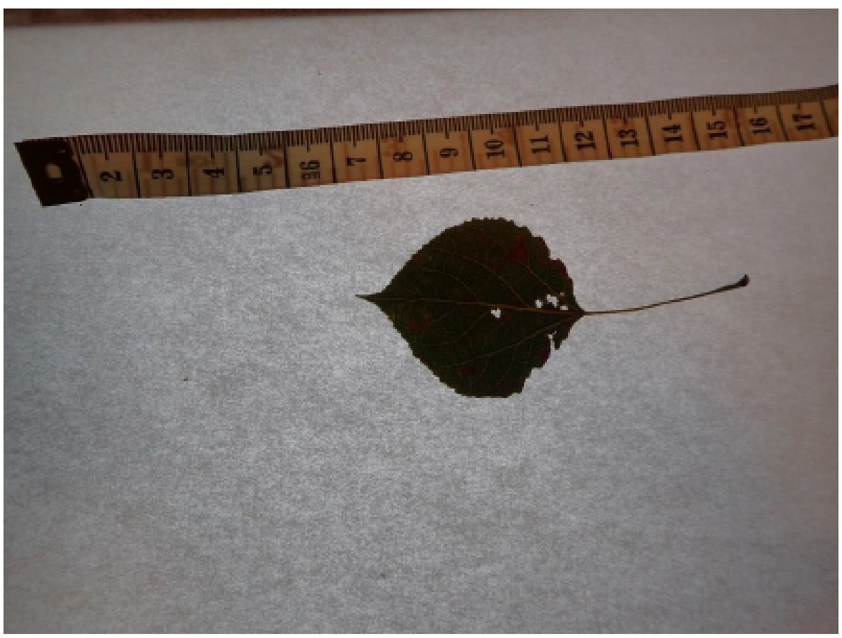
Leaf 1

Leaf 2



Leaf 3

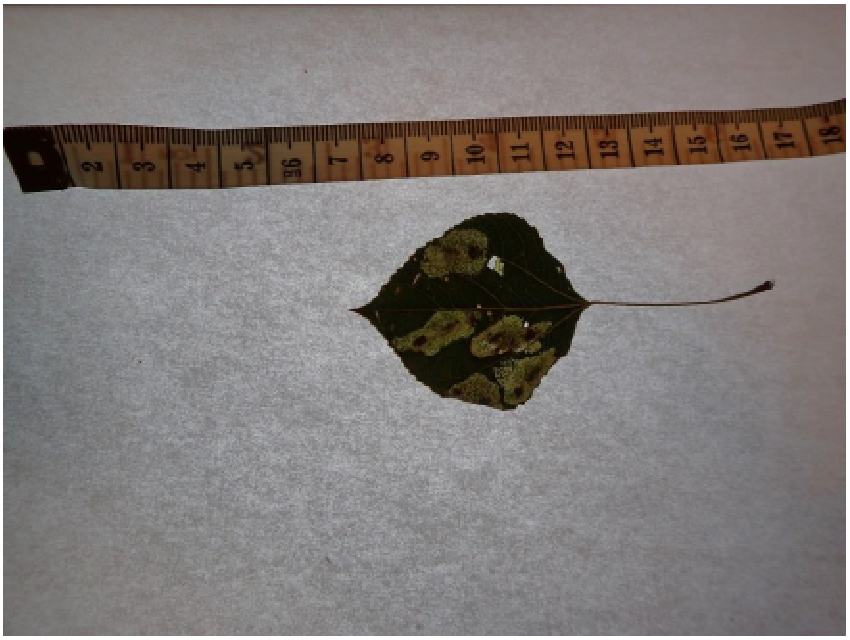
Leaf 4



Leaf 5

Leaf 6

Leaf 7



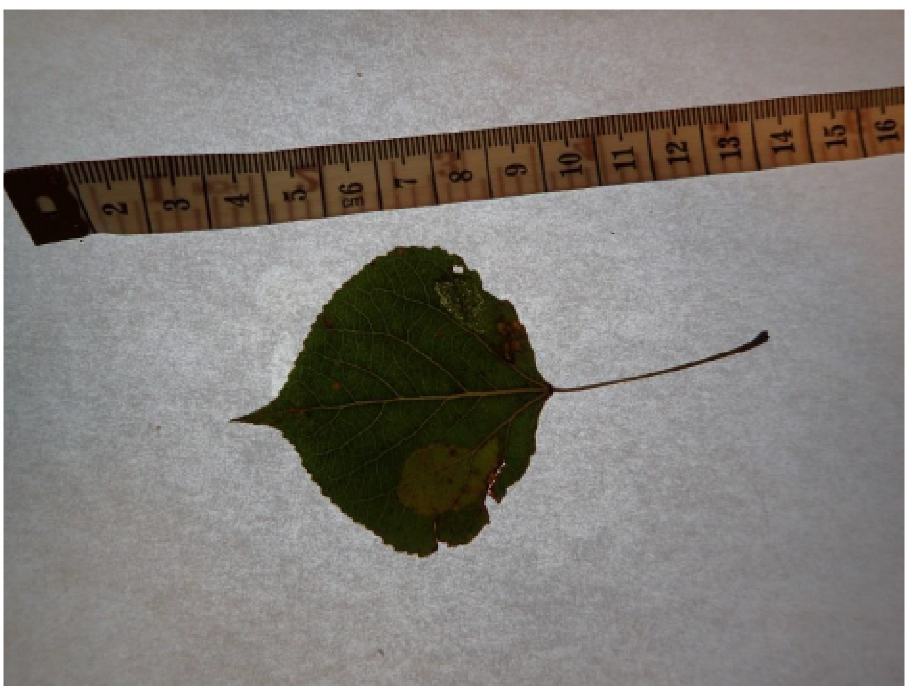
Leaf 8

Leaf 9

Leaf 10

Leaf 11

Leaf 12

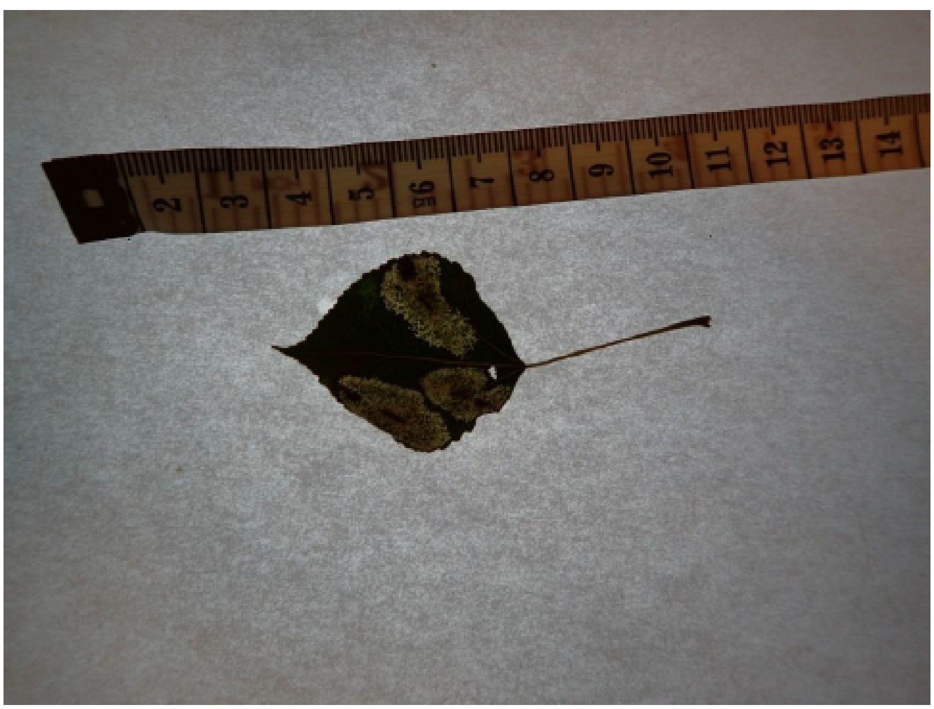


Leaf 13

Leaf 14

Leaf 15

Leaf 16



Leaf 17

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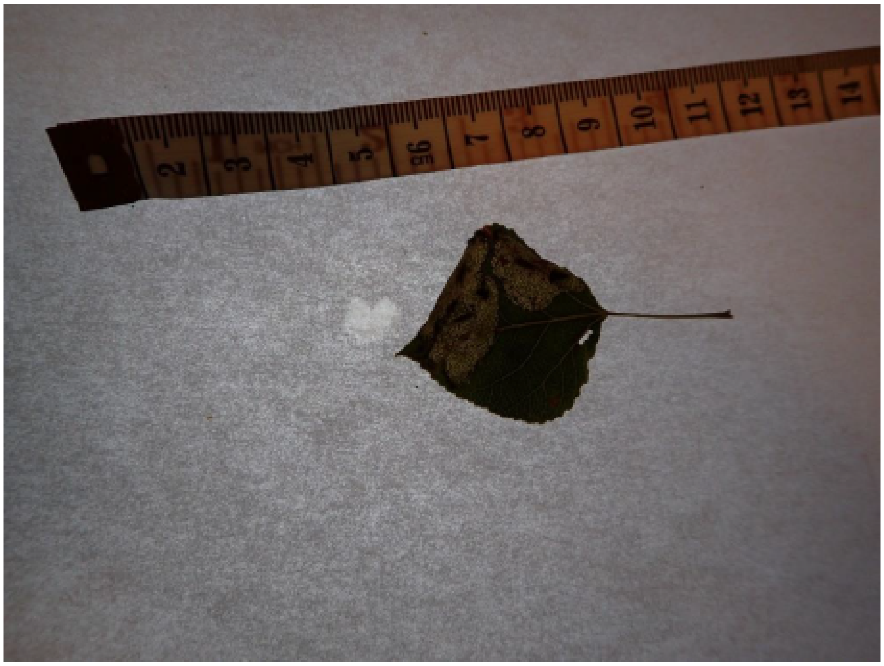
Leaf 21

Leaf 22

Leaf 23

Leaf 24

Leaf 25



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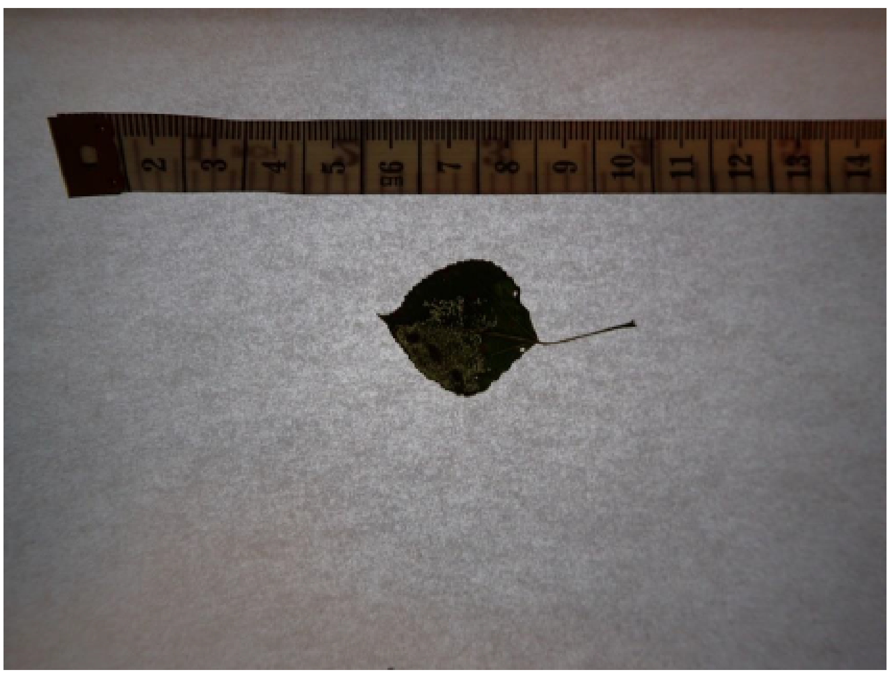

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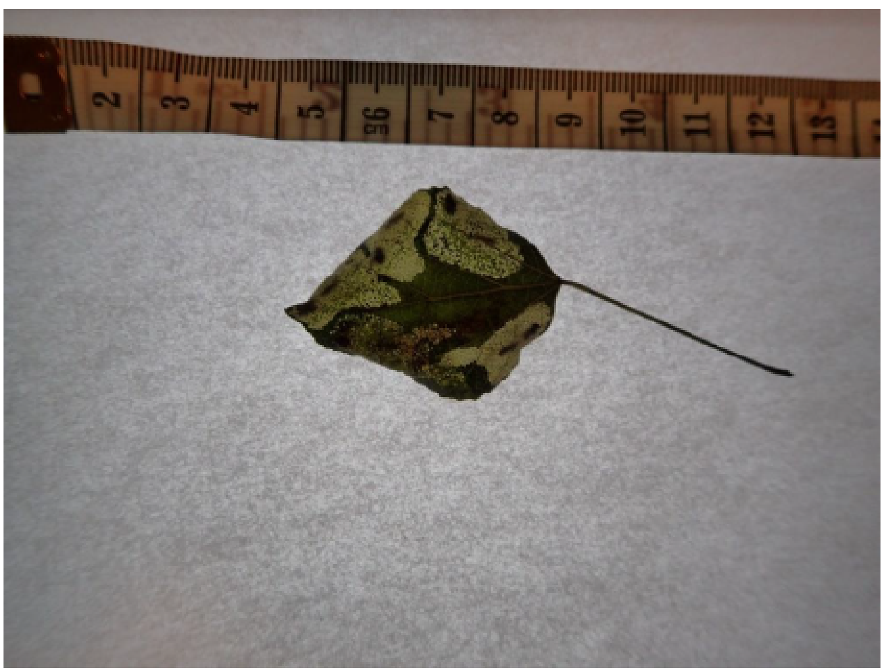

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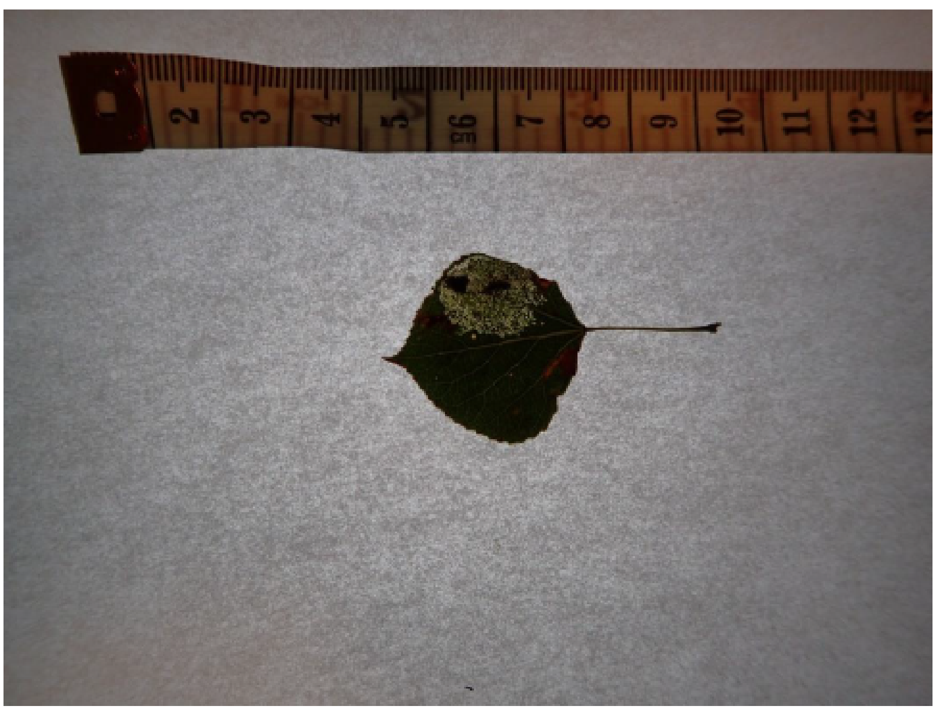

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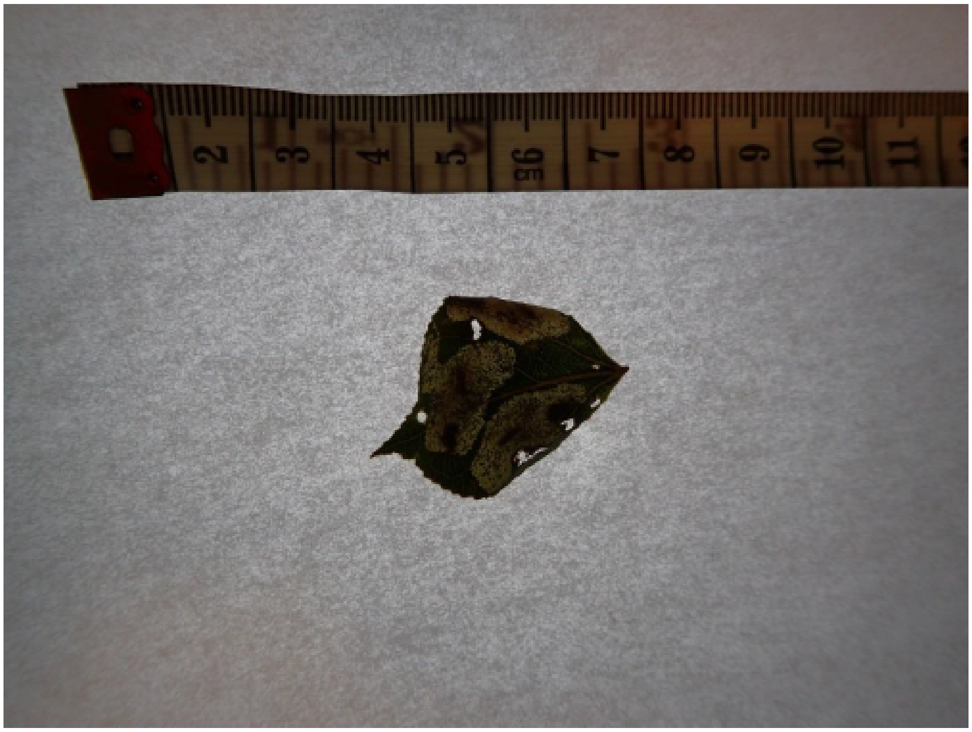
Leaf 33

Leaf 34

Leaf 35



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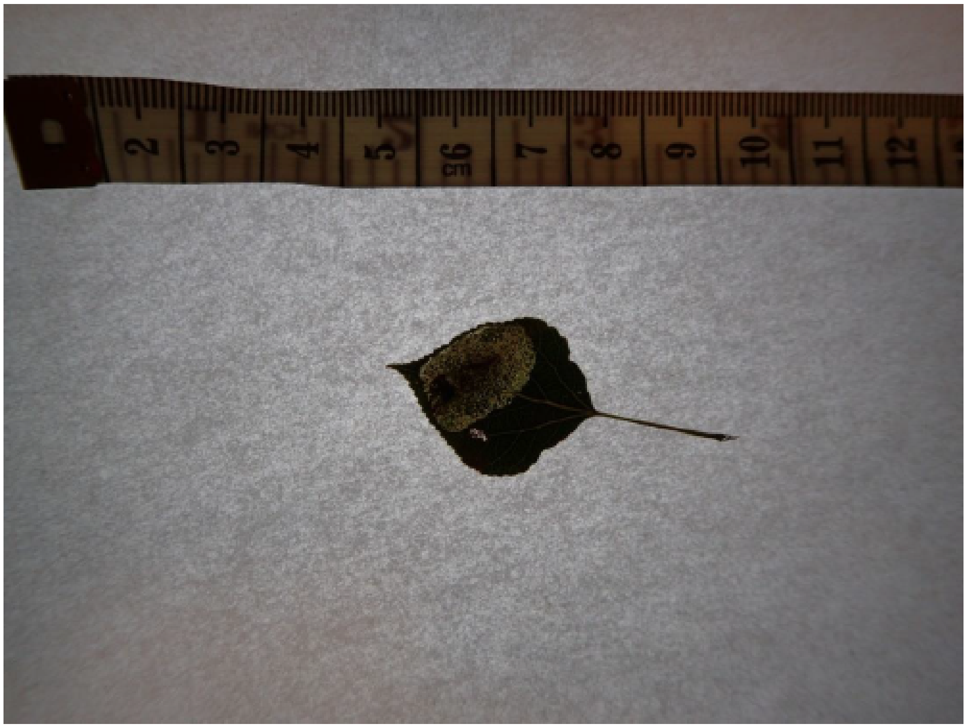
Leaf 38

Leaf 39



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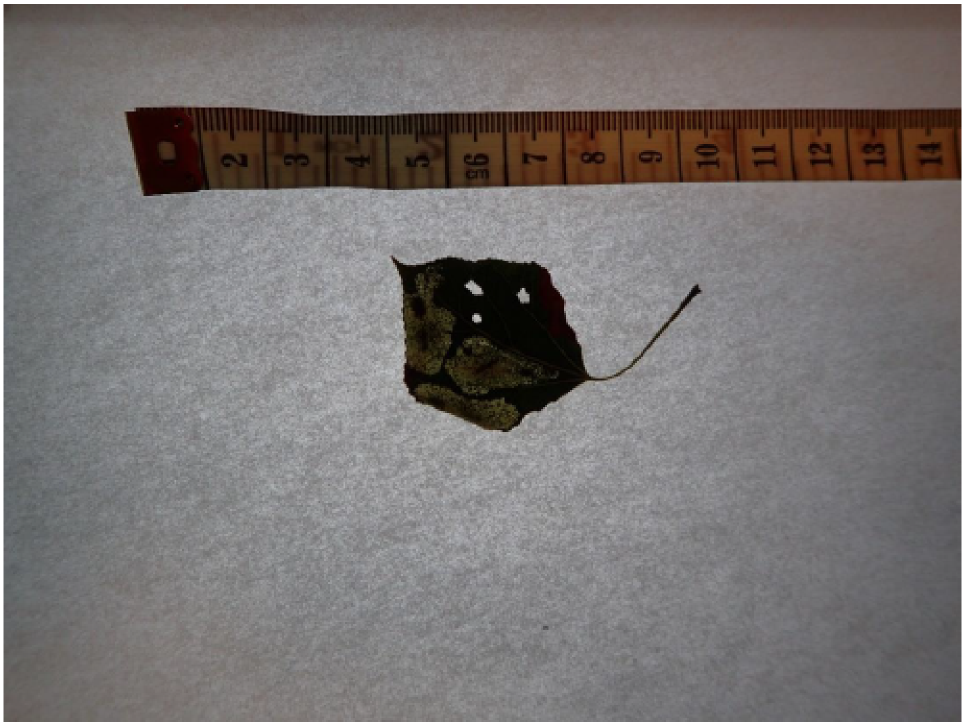

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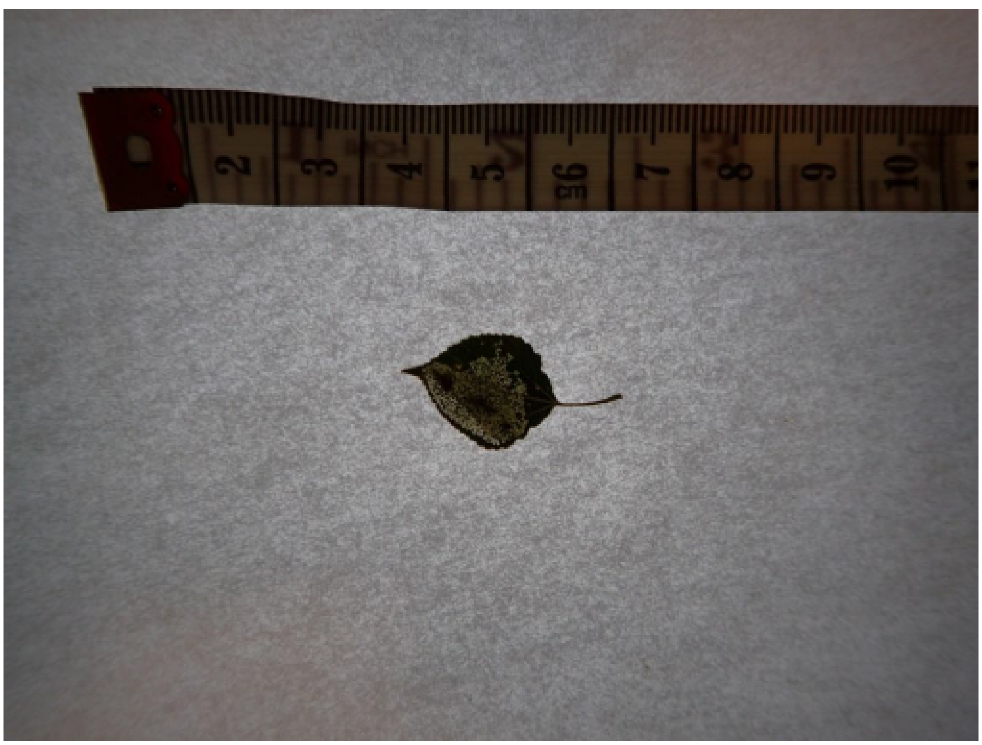

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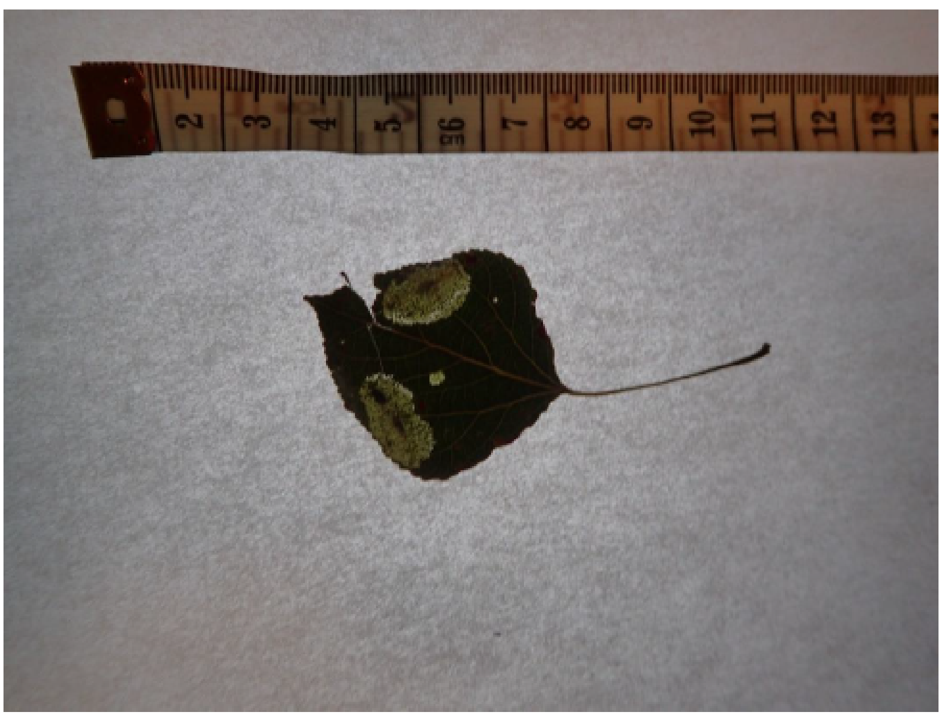

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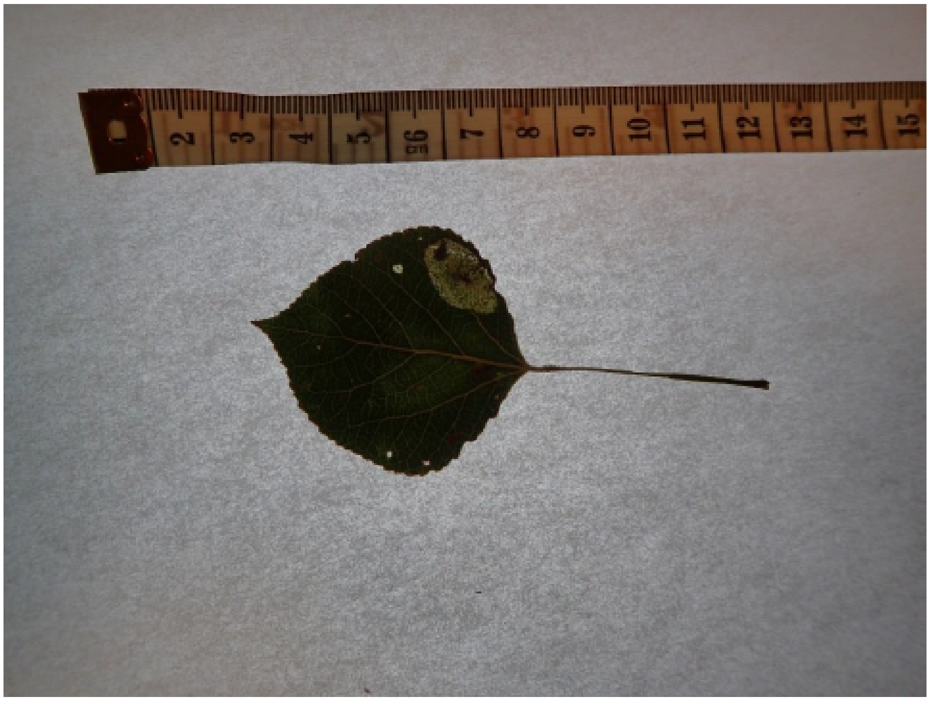

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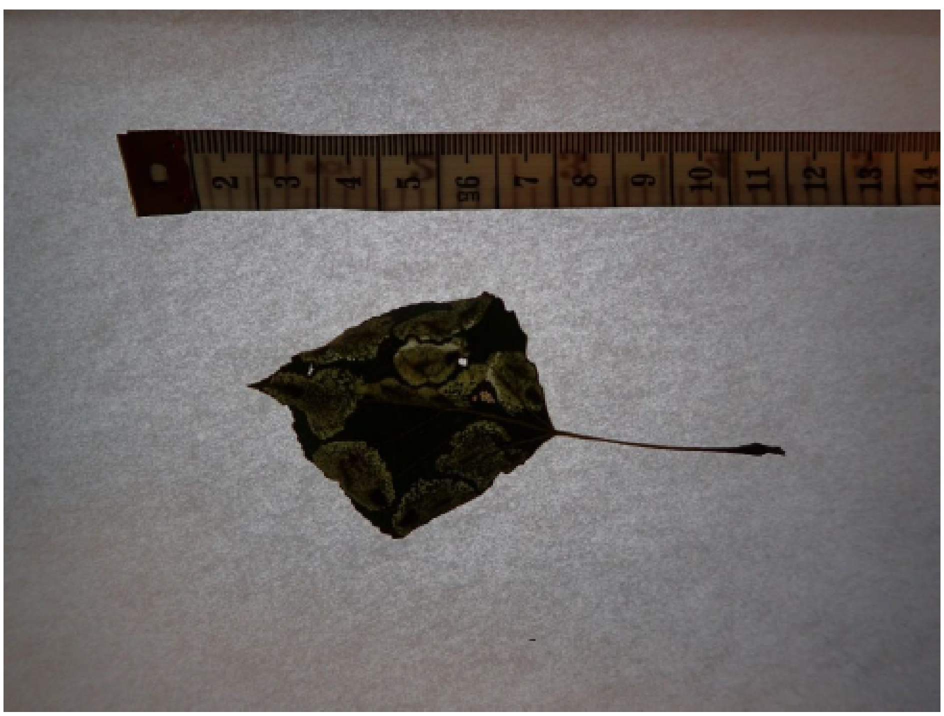
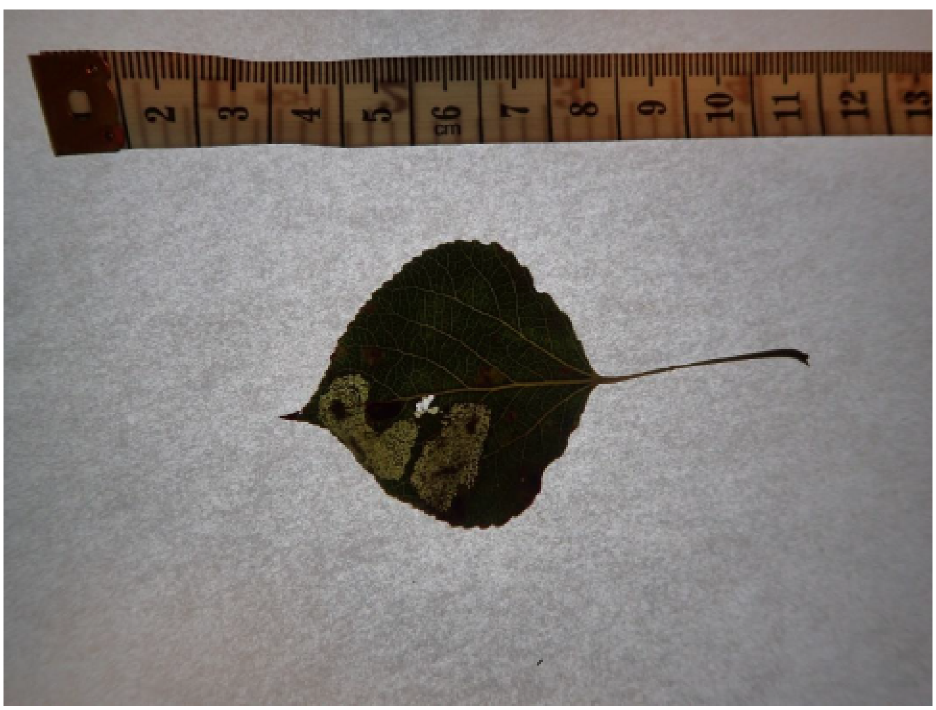


Leaf 47

Leaf 48



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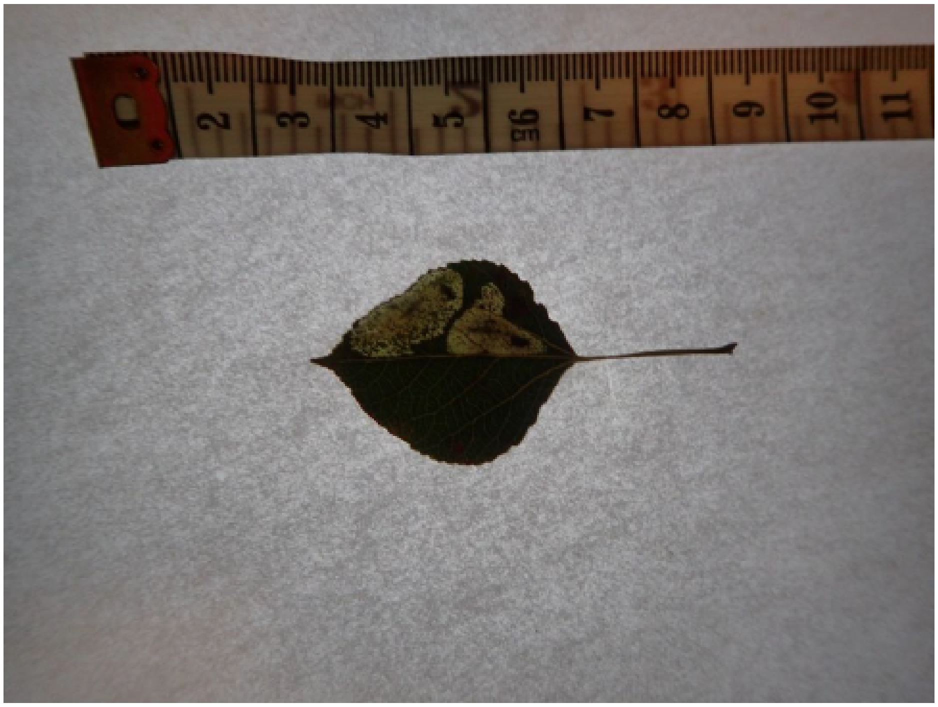

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