

ABSTRACT

Stigmella multispicata was only recently described in 2014 in Primorye Russia by Lithuanian specialists Agne Rociene and Jonas Stonis. This species had been previously observed, in 2010 and later but never named (Van Nieuwerkerkin et al. 2018).

S. multispicata had only been observed in the leaves of the Siberian Elm (*Ulmus pumila*). Recently at the University of Massachusetts Amherst, Dr. Brewer discovered the presence of *S. multispicata* in the leaves of the American Elm (*Ulmus americanus*). With no information on how *S. multispicata* will affect the already weakened *U. americanus* further research is required. We propose to verify the presence of *S. multispicata* in *U. americanus* or any other species of *Ulmus* genus. This study will be conducted on the campus grounds of the University of Massachusetts Amherst by randomly selecting trees from the *Ulmus* genus based on species and location. Infested and uninfested leaves will be collected for study and comparison, and larvae that are found will be grown into their adult form by the field expert Charles Eiseman in order to verify the leafminers identity. If this is in-fact an example of host switching by *S. multispicata* then we should monitor this in order to prevent the

loss of the *U. americanus*. The loss of a species can have detrimental effects on the surrounding ecosystems.

SPECIFIC AIMS

The goal of this project is to sample Elm trees on campus and to look for leafminer activity. Once we have found trees that appear to have leaves affected by leafmines we will take a leaf and identify the type of leafminer that potentially made the track by using the dichotomous keys in *Leafminers of North America* (Eiseman 2019) book.

See if we find evidence of 'host switching' of *S.multiplicata*

Take random leaf samples from elm trees on campus. There are eighteen identified species of Elm trees on the University of Massachusetts, Amherst campus. We will randomly collect leaf specimens from the elm trees. We believe we will find evidence of *S.multiplicata* leafmines.

Identify which leaves have leafminer activity evident on them.

After we randomly sampled the elm trees on the UMASS campus, we will look for the leaves that show evidence of leafminer activity and work to classify what kind of leafminer may have fed on the leaf. There are several types of leafminers that we have found evidence of on campus. We believe that the Elm trees will show leafminer activity.

Use the dichotomous keys to identify the type of leafminer who was feeding on the elm tree see if we find evidence of *S.*

***multispicata* leaf mines.** We are going to classify and label the type of leafminer that has been feeding on the mesophyll of the elm tree leaves. We believe that there be might evidence of host switching going on with the elm tree leaf that Dr. Brewer found.

Collect larval samples if possible to see if the larva grow to be *S.multiplicata*.

Generally, the successful colonization of a novel host by an herbivorous insect is affected by a number of factors, including phylogenetic relatedness of the novel plant to its original host plant (e.g., Odegaard et al., 2005; Ness et al., 2011; Karolewski et al., 2014), t

BACKGROUND

Leafminers are an invasive species that have gone mostly undetected for decades. Historically, leafminers have caused plants and trees to have reduced growth and weakened them so that they are less able to defend themselves from other invaders.

These leafminers have no known natural predators in this area to help control their population growth. Without the help of another species to help control the population of these leafminers, there is concern that the trees affected by these leafminers could die.

Stigmella multispicata is a recently described species of leafminer that has historically only been observed on Siberian Elm trees. *S. multispicata* was first noticed in 2010 by a man doing light collecting in his yard. Since then it has been linked by DNA barcoding around North America and Asia and has been identified by Stonis and Roecine in 2014.

Recently, Dr. Brewer found a leaf on an American Elm tree near the bus stop. When Dr. Brewer took this leaf to Charles Eiseman, a specialist in the field (Author of Leafminers of North America, Master of Science in Botany) he was told it looked like the leaf had been mined by *S. multispicata*. This was an unusual finding because *S. multispicata* usually feed on siberian elm trees not American elms.

This is a recently identified species and not much is known about the impact these leafminers will have if they are feeding on American Elm trees.

In this proposal, we aim to discover whether or not there is evidence of *S. multispicata* host switching from the Siberian Elm to the American Elm.

RESEARCH DESIGN

We will collect leaf samples from the trees on campus by grouping them together according to location and species in an Excel Spreadsheet. Numbers will then be assigned to each tree on campus and we will use a random number generator from excel to randomly select the trees we will sample from. We will group the trees according to species and generate 1 random number for every 5 trees of the same species, use baggies to collect specimens and label with corresponding tree number. We expect to get a random sampling that will represent the majority of trees on campus. We will go out and collect leaf specimens from our randomly selected trees. We will search for leafmines on the leaves we collect. Once we find evidence of leafmines we will use the dichotomous keys from The North American Guide to Leafminers to identify what kind of leafminer activity that we are seeing. Some of the categories we will look at include whether or not there is frass. We are also looking at whether or not we can find a pupal case within the leaf. Identifying the shape, width and length (USE APPENDIX? APPEND dichotomous keys). Use microscope to look better at specimen. We assume that we will find leafminer activity from at least one type of leafminer. (Clustered in one area?)

If we use the microscope and identify larva that appears to be *S.multispicata*, we will attempt to raise the larva to adulthood to see if it is in fact *S.multispicata*. If we are able to raise a larva to maturity, we will ship the specimen to Charles Eiseman to identify the species.

IMPACT AND SIGNIFICANCE

When a species is lost, it can have a detrimental effect on the surrounding ecosystem. The discovery of *S.multispicata* on American Elms would be unprecedented. There is little research available for *S. multispicata* and its effects on nature. There is no research to date on *S.multispicata* feeding on American Elm trees. It is of utmost importance to study these newly identified larvae and their effects on the ecosystem. If they are able to host switch to an American Elm it raises the possibility of the balance of nature being thrown off and creating a ripple effect that could negatively impact other species and wildlife. Finding evidence of *S. multispicata* could help us to be prepared and educated on this species before it has a chance to get out of control and throw off the balance of nature.

REFERENCES

Moreira ín, Abdala-Roberts L, Mier y Teran JCBerny, Covelo F, de la Mata úl, Francisco M, Hardwick B, Pires RMatheus, Roslin

T, Schigel DS, et al. *Impacts of urbanization on insect herbivory and plant defences in oak trees*. Oikos [Internet]. <https://onlinelibrary.wiley.com/doi/epdf/10.1111/oik.05497>

Kirichenko N, Augustin S, Kenis M. *Invasive leafminers on woody plants: a global review of pathways, impact, and management*. Journal of Pest Science [Internet]. 2019 ;92(1):93 - 106.

Available from:

<http://link.springer.com/10.1007/s10340-018-1009-6><http://link.springer.com/content/pdf/10.1007/s10340-018-1009-6.pdf><http://link.springer.com/article/10.1007/s10340-018-1009-6/fulltext.html><http://link.springer.com/content/pdf/10.1007/s10340-018-1009-6.pdf>

WALCZAK U, BOGDZIEWICZ M, ZYTKOWIAK R, KAROLEWSKI P, BARANIAK E.

Maladaptive host choice by an alien leaf miner Phyllonorycter leucographella (Lepidoptera: Gracillariidae) has the potential to limit its invasiveness. European Journal of Entomology [Internet]. 2018 ;115:318 - 325.

Available from:

<http://www.eje.cz/doi/10.14411/eje.2018.031.pdf>

van Nieukerken EJ, Gilrein DOWen, Eiseman CS, van Nieukerken EJ, Gilrein DOWen, Eiseman CS, van Nieukerken EJ, Gilrein DOWen,

Eiseman CS, van Nieukerken EJ, et al. *Stigmella multispicata* Rociene. & Stonis, an Asian leafminer on Siberian elm, now widespread in eastern North America (Lepidoptera, Nepticulidae). ZooKeys [Internet]. 2018 ;7845043182332661288113235122124124:95 - 125. Available from: <https://zookeys.pensoft.net/articles.php?id=27296>

Bernardo U, van Nieukerken EJ, Sasso R, Gebiola M, Gualtieri L, Viggiani G. *Characterization, distribution, biology and impact on Italian walnut orchards of the invasive North-American leafminer Coptodisca luciflua* (Lepidoptera: Heliozelidae). Bulletin of Entomological Research [Internet]. 2015 ;105(2):210 - 224. Available from: https://www.cambridge.org/core/product/identifier/S0007485314000947/type/journal_article

STONIS JONASR, ROCIENÈ AGNÈ. *Additions to the Nepticulidae (Lepidoptera) of East Asia, with descriptions of three new species from Primorskiy Kray*. Zootaxa [Internet]. 2014 ;3846(2):204. Available from: <http://biotaxa.org/Zootaxa/issue/view/zootaxa.3846.2>

éré C, Augustin S, Turlings TCJ, Kenis M. *The invasive alien leaf miner Cameraria ohridella and the native tree Acer*

pseudoplatanus: a fatal attraction?. Agricultural and Forest Entomology [Internet]. 2010

;1213339415252659371235514127997131103711115721174229172435(2):151 - 159. Available from:
<http://blackwell-synergy.com/doi/abs/10.1111/afe.2010.12.issue-2>

SINCLAIR ROBYNJEAN, HUGHES LESLEY. *Leaf miners: The hidden herbivores*. Austral Ecology [Internet]. 2010 ;35(3):300 - 313. Available from:
<https://doi.org/10.1111/j.1442-9993.2009.02039.x>

Nardini A, Raimondo F, Gullo MALo, Salleo S. *Leafminers help us understanding leaf hydraulic design*. Plant, Cell & Environment [Internet]. 2010 . Available from:
<http://doi.wiley.com/10.1111/j.1365-3040.2010.02131.x>

Agosta SJ. *On ecological fitting, plant-insect associations, herbivore host shifts, and host plant selection*. Oikos [Internet]. 2006 ;114(3):556 - 565. Available from:
<http://blackwell-synergy.com/doi/abs/10.1111/oik.2006.114.issue-3>

Lee DHyung, Park J-J, Cho K. Characterization of Leaf Mining Damage of *Liriomyza trifolii* (Diptera: Agromyzidae) in Cherry-Tomato Greenhouse. Journal of Asia-Pacific Entomology

[Internet]. 2004 ;7(2):201 - 205. Available from:
<https://linkinghub.elsevier.com/retrieve/pii/S12268615086021>

62

Miller F. The Elms Insect Resistance of Elm Genotypes. 2nd ed.
(Dunn CP). Boston, MA: Springer US; 2000 pp. 137 - 154. Available
from: http://doi.org/10.1007/978-1-4615-4507-1_8

Hubbes M. The American elm and Dutch elm disease. The Forestry
Chronicle [Internet]. 1999 ;75(2):265 - 273. Available from:
<http://pubs.cif-ifc.org/doi/10.5558/tfc75265-2>

Miller Apape. Leaf-Mining Insects and Fluctuating Asymmetry in Elm
Ulmus glabra Leaves. The Journal of Animal Ecology [Internet].
1995 ;64(6):697. Available from:
<https://www.jstor.org/stable/5849?origin=crossref>

Reavey D, Gaston KJ. The Importance of Leaf Structure in
Oviposition by Leaf-Mining Microlepidoptera. *Oikos* [Internet].
1991 ;61(1):19. Available from:
<https://www.jstor.org/stable/3545403?origin=crossref>

Auerbach M, Simberloff D. Rapid Leaf-Miner Colonization of
Introduced Trees and Shifts in Sources of Herbivore Mortality.
Oikos [Internet]. 1988 ;52(1):41. Available from:
<https://www.jstor.org/stable/3565980?origin=crossref>

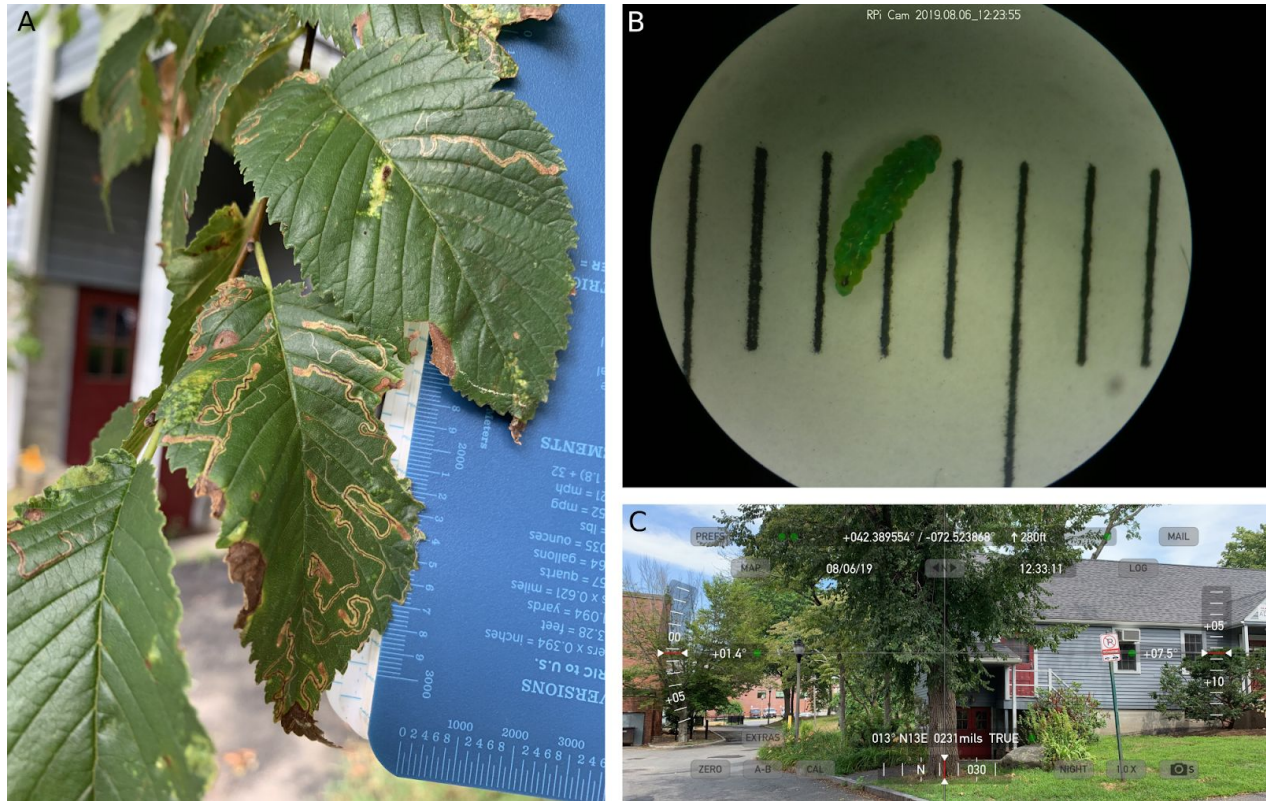


Figure 1: Leafminer activity spotted on an *U.americanus*.

A. Leafmines from *S.multispicata* on an American Elm leaf.B.

Larval sample taken from *U.americanus* distance is marked with 1 mm between lines. C. *U.americanus* tree where leafminer activity and *S.multispicata* larva were found.

We want to have something about host switching in the BACKGROUND of this paper. Because host switching is a good part of why we want to do this research. There are concerns if these leaf miners are host switching they could damage the Elm trees and they don't have many natural predators around. The physiological similarities between the preferred host and the new host are likely to determine whether or not host switching occurs. The length of time that the insect and the new source of food are in contact also can help determine whether or not host switching occurs. If the two different food sources for the insect are phylogenetically related then there is a greater chance for host switching.

Leafminer ID strategies

Is there frass?

Pupal Case? Cut from mine?

Origin of mine

Linear or linear blotch

Cocoon or not cocoon within the mine

(color of cocoon if there is one)

Location of slit

Shape of mine

Width of mine

Length of mine

Curvatures of mine

Depth or area of mine

Timing of change in width

Abrupt or gradual change in width

Where deposited upper or lower leaf surface

Color of larvae

Is there larvae

Appearance of frass

Can we see eggshell

Materials:

Microscope

Not going to sample from the spp elm pool since they are not identified.

Will take random samples from each species of elm. Computer generated (preferable).

Categorize with a 1 or 0 whether or not leafminer activity is observed.

Take a photograph of tree and photographs of observed leafminer activity

Remove a sample leaf from the tree. (Log tree location, and species surrounding activity)

Place the sample in a bag and label the bag with name of tree and coordinates of location.

Use the dichotomous keys to identify the type of leafminer activity we are seeing. Group similar leafminer activity together.

We are sampling any leafminer activity that we see from a variety of Elm trees on campus and then we are seeing which leafmines look like *stigmella multispicata*. Then when we find the leafmines that look like *stigmella multispicata*. Then we will see which trees *multispicata* come from. Characterize what the leafmines look like and come up with some statements on what the galleries are like after looking at them and see if the leafminer tracks vary depending on the type of tree it is.

Stigmella Multispicata is a newer species of leafminers in this area. Historically, they have been found to feed on Siberian Elm trees.

A unique feature that sets the larva apart is the fact that the *S. multispicata* is green.

Background information

- Leafminers are an invasive species
- *S. multispicata* was first recognized in 2014
- Reduce growth in trees, can weaken trees (young trees are most susceptible) allowing for fungal invaders to gain a foothold.
- Generally do not kill host plants; affects aesthetics

Kirichenko, N., Augustin, S. & Kenis, M. J Pest Sci (2019) 92: 93.

<https://doi.org/10.1007/s10340-018-1009-6>

- Opening in the leaf can allow other forin invaders in.
- *S. multispicata* has no predators in the US that we know of so the potential for rapid infestation is very possible.
- *S. multispicata* leafmines are characterized by the egg placement on the leaf underside in vein axils, larval exit on leaf underside, and green to blue-green larval color.

Assign a random ID number to each tree. Use a random number generator to choose which trees to sample from.

Characteristics of stigmella multispicata larva

- All mines start in the leaf vein axils
- Green larve (blue/green)

- Exit from lower leaf epidermis
-

SPECIFIC AIMS

The goal of this project is to sample Elm trees on campus and to look for leafminer activity. Once we have found trees that appear to have leaves affected by leafmines we will take a leaf and identify the type of leafminer that potentially made the track by using the dichotomous keys in *Leafminers of North America* (Eiseman 2019) book.

See if we find evidence of 'host switching' of *S.multiplicata*

Take random leaf samples from elm trees on campus. There are eighteen identified species of Elm trees on the University of Massachusetts, Amherst campus. We will randomly collect leaf specimens from the elm trees. We believe we will find evidence of *S.multiplicata* leafmines.

Identify which leaves have leafminer activity evident on them. After we randomly sampled the elm trees on the UMASS campus, we will look for the leaves that show evidence of leafminer activity and work to classify what kind of leafminer may have fed on the leaf. There are several types of leafminers that we have found evidence of on campus. We believe that the Elm trees will show leafminer activity.

Use the dichotomous keys to identify the type of leafminer who was feeding on the elm tree see if we find evidence of *S. multispicata* leaf mines. We are going to classify and label the type of leafminer that has been feeding on the mesophyll of the elm tree leaves. We believe that there be might evidence of host switching going on with the elm tree leaf that Dr. Brewer found.

Collect larval samples if possible to see if the larva grow to be *S.multiplicata*.

Generally, the successful colonization of a novel host by an herbivorous insect is affected by a number of factors, including phylogenetic relatedness of the novel plant to its original host plant (e.g., Odegaard et al., 2005; Ness et al., 2011; Karolewski et al., 2014), t

RESEARCH DESIGN

We will collect leaf samples from the trees on campus by grouping them together according to location and species in an Excel Spreadsheet. Numbers will then be assigned to each tree on campus and we will use a random number generator from excel to randomly select the trees we will sample from. We will group the trees according to species and generate 1 random number for every 5 trees of the same species. Use baggies to collect specimens and label with corresponding tree number. We expect to get a random sampling that will represent the majority of trees on campus.

We will go out and collect leaf specimens from our randomly selected trees. We will search for leafmines on the leaves we collect. Once we find evidence of leafmines we will use the dichotomous keys from The North American Guide to Leafminers to identify what kind of

leafminer activity that we are seeing. Some of the categories we will look at include whether or not there is frass. We are also looking at whether or not we can find a pupal case within the leaf. Identifying the shape, width and length (USE APPENDIX? APPEND dichotomous keys). Use microscope to look better at specimen. We assume that we will find leafminer activity from at least one type of leafminer. (Clustered in one area?)

If we use the microscope and identify larva that appears to be *S.multispicata*, we will attempt to raise the larva to adulthood to see if it is in fact *S.multispicata*. If we are able to raise a larva to maturity, we will ship the specimen to Charles Eiseman to identify the species.

s there frass?

Pupal Case? Cut from mine? Cocoon or not cocoon within the mine

Origin of mine Linear or linear blotch

Shape of mine Width of mine Length of mine Curvatures of mine

(color of cocoon if there is one)

Location of slit

Depth or area of mine

Timing of change in width

Abrupt or gradual change in width

Where deposited upper or lower leaf surface

Color of larvae

Is there larvae

Appearance of frass

Can we see eggshell

Insects are more able to feed on a new kind of plant if the new plant is genetically similar to their preferred food source.

Leafminers are a taxonomically diverse group of endophagous insects. A number of them are pests in forestry, horticulture and agriculture, and some of them have become important invasive species (Krichenko, Augustin)

Leafminers are a unique group of plant eating insects that have the potential to become pests and invasive species.

Leafminers can travel into other continents via goods being shipped around the world.

Leafminers can cause significant damage to trees. (I don't know how accurate this statement is. Everything that I have read suggests that it is very rare for leafminers to kill the host plant. It can weaken the tree, and reduce its growth)

Kirichenko, N., Augustin, S. & Kenis, M. J Pest Sci (2019) 92: 93.

<https://doi.org/10.1007/s10340-018-1009-6>

Abstract (Outline)

Background Info:

- Leafminers are an invasive that can damage commercial crops: (Leaf miners do not normally kill the host plant. However, they can weaken the plant (speculated ?), lowering its defences allowing a pathway for other invaders to take hold of the tree). *We suspect that trade of live plants through nurseries played a role in the sudden spread of this invasive species* (Nieukerken et al. 2018)
- More from Europe than Asia in North America ([Kirichenko](#) et al. 2019)
- Ornamental trees with leafmines are not aesthetically pleasing
- Poorly studied (Almost all of the articles that I have read mention this)
- *S. multispicata* only recently described (Rociene, Stonis, 2014) Collected in 2010 by James Vargo in Indiana but never identified.

Statement of need

- Not much information on *S. multispicata*
- How many species of elm have this leafminer infected

Proposal objective

- Document *S. multispicata* feeding on elm's other than Siberian
- Add to the scientific community's knowledge base about this invasive

General Strategy

- Randomly select Elm trees to sample based on location and species
- ID Leaf miner activity (on tree and surrounding vegetation)
- Collect samples (Infected or not)

- Determine the species of leafminer
- Correlation?

Significance

- Our research may add to the data on this species of leafminer
- is this species becoming a generalist (not feeding/breeding on specific species)

What are the natural predators of *stigmella multispicata*? Wasp *Diglyphus isaea* ?

IMPACT AND SIGNIFICANCE

When a species is lost, it can have a detrimental effect on the surrounding ecosystem. The discovery of *S.multispicata* on American Elms would be unprecedented. There is little research available for *S. multispicata* and its effects on nature. There is no research to date on *S.multispicata* feeding on American Elm trees. It is of utmost importance to study these newly identified larvae and their effects on the ecosystem. If they are able to host switch to an American Elm it raises the possibility of the balance of nature being thrown off and creating a ripple effect that could negatively impact other species and wildlife. Finding evidence of *S. multispicata* could help us to be prepared and educated on this species before it has a chance to get out of control and throw off the balance of nature.