Reduced Function in *Chamaenerion angustifolium* after Sub-Lethal Glyphosate Exposure

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What is glyphosate?

- Glyphosate is the active ingredient of many broad-spectrum herbicides.
- Used in forestry operations to control competitive plant species, predominantly trembling aspen (*Populus tremuloides*).
- As a result of aerial applications, non-target forest understory plants receive a sublethal dose and develop abnormalities
  - 90% of treatments of glyphosate-based herbicides (GBH) are applied aerially.

Image from: http://stopthespraybc.com/

What is glyphosate?

- Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) of the shikimate pathway

- Produces aromatic amino acids (phenylalanine, tyrosine, and tryptophan) for plant growth and other biochemical processes.

- How does this impact the reproduction of forest understory plants and biocommunication between plants and insect pollinators?

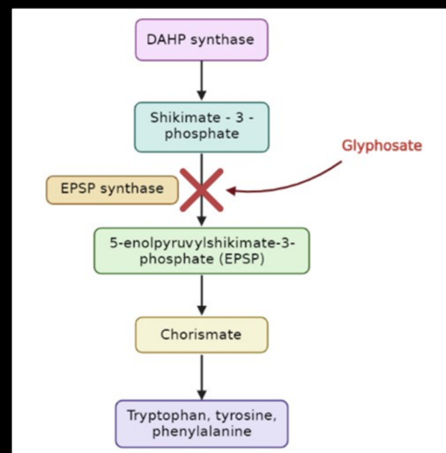

Image by: A. Golt

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Study Species: Fireweed (*Chamaenerion angustifolium*)

- Prominent herb in northern BC.
- After a disturbance, *C. angustifolium* is one of the primary successional pioneer species.
  - Recycles available nutrients through its own dead and decaying plant matter, thereby improving soil quality for other plant species during early succession.
- Fireweed has been used by Indigenous people for food

Image from: https://www.alaskawildland.com/fireweed/

Rationale

- High prevalence of GBH worldwide.

- Understand changes to the beneficial ecological and cultural services provided by fireweed.

- Two Studies:
  - **Growth chamber experimentation:** we conducted controlled experimentation using fireweed, to better understand this species' growth and reproductive potential.
  - **Operational study 2021 – 2022:** We collected samples of fireweed from operationally managed forest stands one and two years after GBH applications took place.

- Aimed to contribute to the knowledge base on how plant systems function

Image from: https://www.alaskawildland.com/fireweed/

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Objectives

1. To quantify the reproductive capacity of fireweed flowers post GBH application.
   - Explored pollen viability and glyphosate residue presence.

2. To quantify the biocommunicative potential of fireweed flowers post GBH application.
   - Explored stamen fluorescence.

Image by: A. Golt

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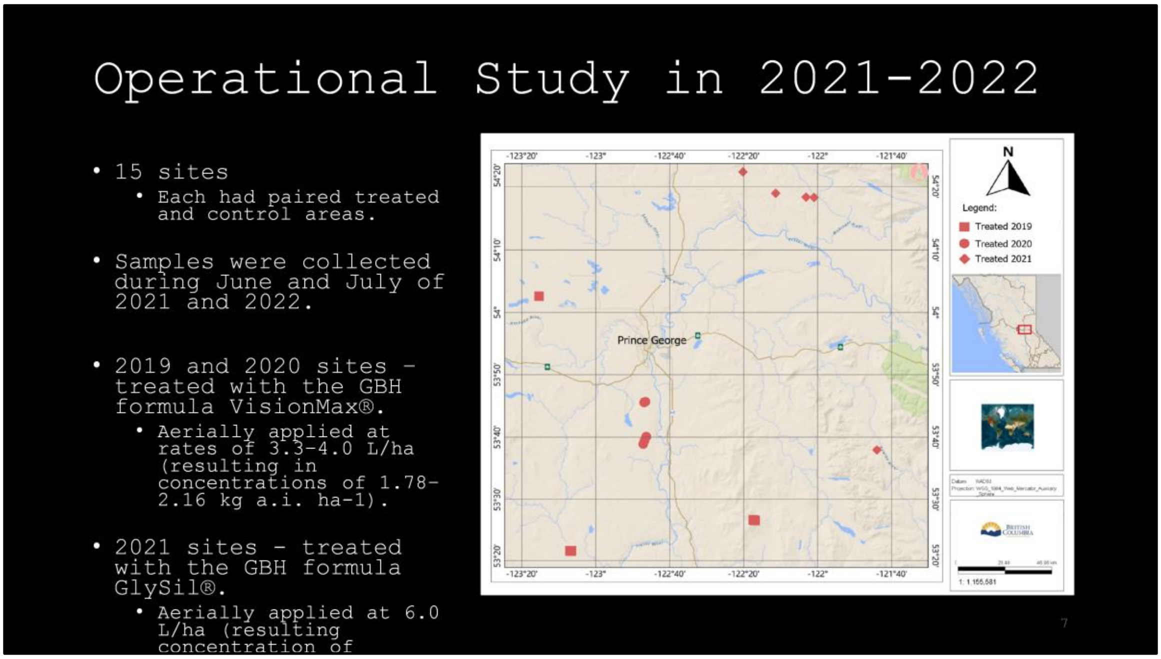

5 sites treated in 2019, 5 sites treated in 2020, and 5 sites treated in 2021.
Each treated site had a paired control site (not treated with GBH) of the same vegetation complex, that was sampled.

There were two formulas applied (VisionMax and Glysil) → we didn't look at adjuvants or surfactants (the other ingredients in the formula) and only analyzed for the active ingredient's glyphosate and metabolite AMPA

The sites were previously logged using a clear-cut method of harvesting, planted with coniferous trees, lodgepole pine (*Pinus contorta* Dougl. ex Loud) and/or hybrid white spruce (*Picea glauca* (Moench) Voss x *engelmannii* Parry ex. Engelm) and treated with a GBH in either 2019, 2020, or 2021 (one-year before sampling) when planted trees were between 5 and 15 years of age.

Parts of these sites were left untreated (pesticide-free zones) due to the presence of streams to prevent glyphosate contamination and run-off. These untreated areas within the sites or other nearby untreated regions with similar vegetation complexes served as experimental controls. Paired treatment and control areas for each site were identified from forest industry operation maps and were confirmed visually on site through marked treatment lines.

Sites were located in the SBSvk, SBSdw2, SBSdw3, and ESSFwk1 biogeoclimatic ecosystem classification (BEC) zones (Table 1).

Operational Study in 2021-2022

- We looked at:
  - Pollen viability as a representation of reproductive capacity.
  - Floral fluorescence as an indicator or biocommunicative potential.
  - Glyphosate residue analysis of floral tissues.

Analytical Test	Treated one-year prior	Treated two-years prior	Controls
Glyphosate Residue	15 (3 replicates from 5 sites)	30 (3–5 replicates from 8 sites)	30 (3–5 replicates from 8 sites)
Fluorescence	15 flowers (5 plants, 3 flowers per plant) per site	15 flowers (5 plants, 3 flowers per plant) per site	15 flowers (5 plants, 3 flowers per plant) per site
Pollen Viability	15 flowers (5 plants, 3 flowers per plant) per site	15 flowers (5 plants, 3 flowers per plant) per site	15 flowers (5 plants, 3 flowers per plant) per site

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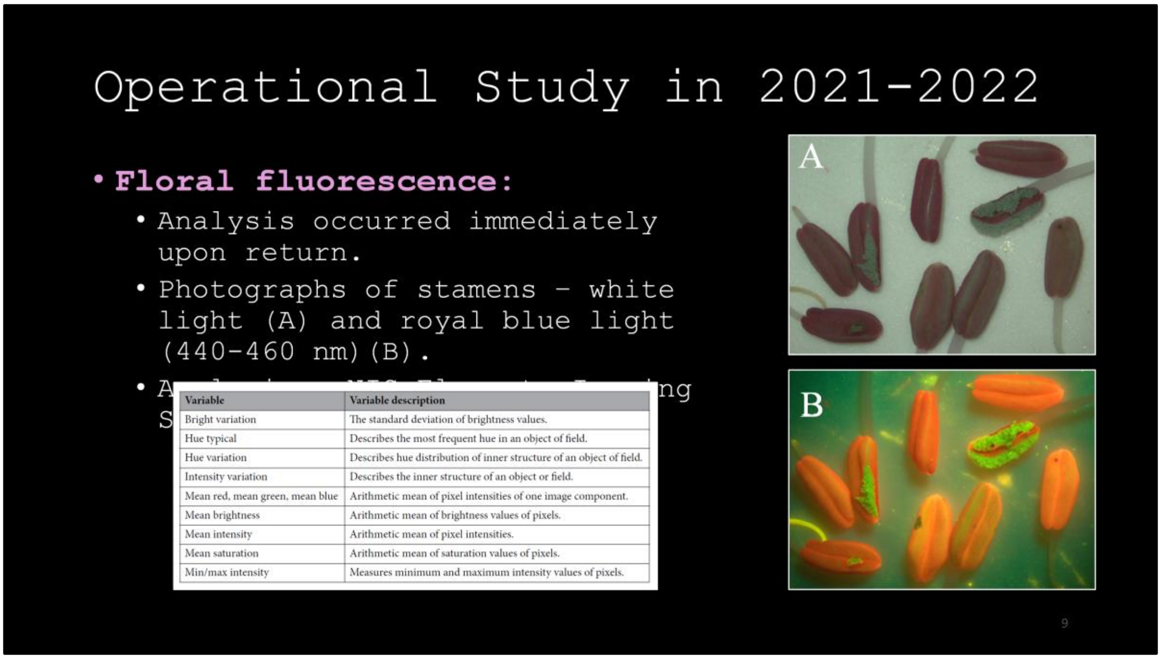
Operational sampling was conducted to determine if the reproduction or biocommunication components of fireweed were interrupted by GBH applications in the real world.

We chose to study pollen viability as a representation of reproductive capacity and floral fluorescence as an indicator or biocommunicative potential.
Table: Composite sample replicate numbers of fireweed (C. angustifolium) flowers tested with each type of analysis.



Variable	Variable description
Bright variation	The standard deviation of brightness values.
Hue typical	Describes the most frequent hue in an object of field.
Hue variation	Describes hue distribution of inner structure of an object of field.
Intensity variation	Describes the inner structure of an object or field.
Mean red, mean green, mean blue	Arithmetic mean of pixel intensities of one image component.
Mean brightness	Arithmetic mean of brightness values of pixels.
Mean intensity	Arithmetic mean of pixel intensities.
Mean saturation	Arithmetic mean of saturation values of pixels.
Min/max intensity	Measures minimum and maximum intensity values of pixels.

Analysis of fluorescence was conducted on 15 flowers per site immediately after returning from the field to avoid floral degradation.

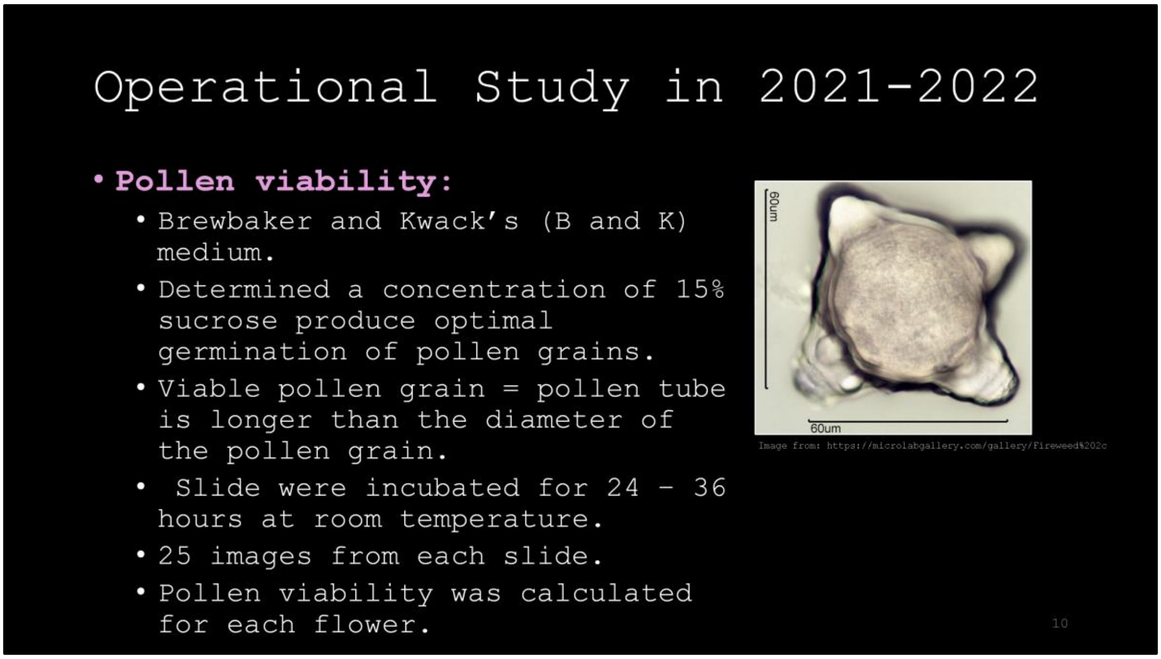
Photographs were taken using a Leica dissecting microscope with digital camera and Zen Lite software.

We used the stereomicroscope adapter system by NIGHTSEA with a royal blue light (wavelength range of 440-460 nm) to induce fluorescence.

Stamens were separated from the rest of the flower and placed under the microscope to capture images with all the anthers in focus. We photographed stamen samples under white light (light emitted from microscope) and under the royal blue fluorescence filter.

Analysis of resulting colour pixels captured by the photography was conducted using NIS-Elements Imaging

Software. The stamen in each image were selected as areas of interest (AOI) and were isolated in the images (measured in pixels2) for a focused analysis. Colour parameters were assessed and calculated for each AOI by the software,



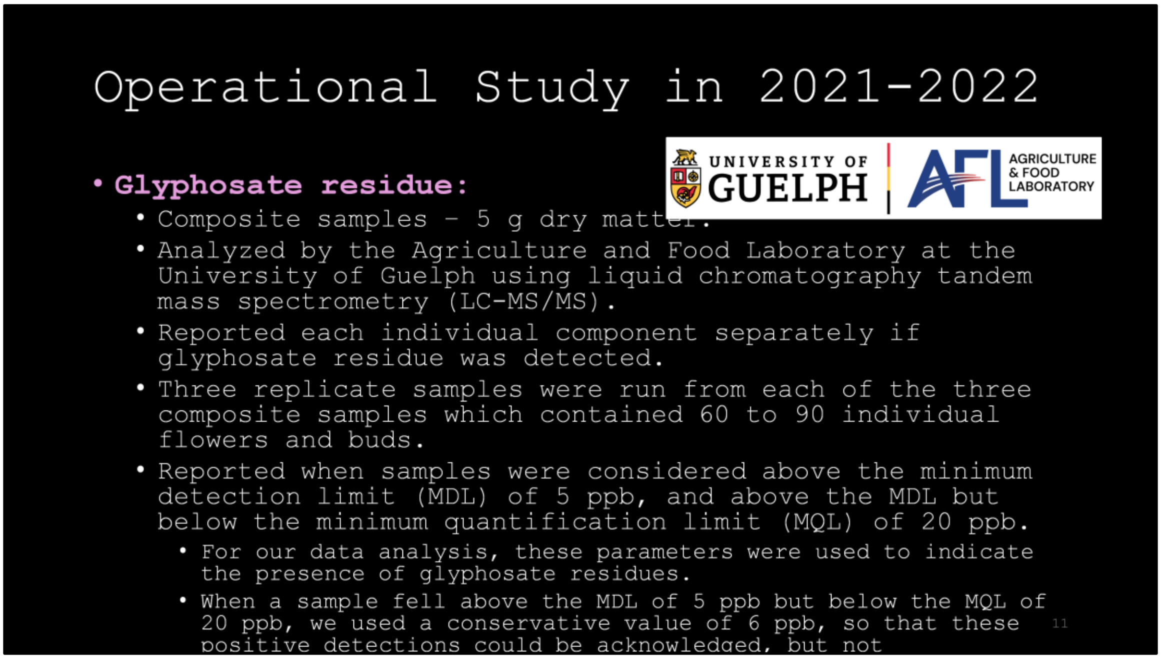
Following the imaging of the fresh flowers for fluorescence analysis, pollen was mechanically removed from anthers and Brewbaker and Kwack's (B and K) medium was prepared for pollen viability testing.

The amount of sucrose required to produce optimal germination of pollen grains varies between plant species. and we were unable to find existing literature on the optimal sucrose content for fireweed pollen germination.
We initially tested the pollen viability of fireweed using varying amounts of sucrose at 5, 10, 15, 20, 30, 40, 50, 60, and 70%.
The optimal concentration of sucrose that induced the highest rate of pollen tube formation in our trial was 15%; therefore, all proceeding viability testing was completed using this concentration of sucrose.

Fresh pollen grains were placed on a depression microscope slide. Two drops of B and K medium were added to each slide and pollen grains were mixed into the media using a toothpick. Each slide contained the pollen grains of one flower. Fifteen slides were prepared per site, representing three replicate flowers from each of five individual replicate plants per site

A cover slip was placed on top of the slide and slides were incubated for 24–36 h at room temperature, in a Petri dish lined with moist filter paper and sealed with parafilm to maintain humidity. Upon completion of the incubation period, slides were observed using an Eclipse FN1 Nikon microscope at 10× magnification. A microscope camera with NIS-Elements Imaging Software was used to view the pollen grains and capture images.

A total of 25 images were captured from each slide in a grid-like manner across the slide moving from the top left corner to the bottom right corner of the slide. The total number of pollen grains per slide were counted, and pollen viability was calculated for each flower and then averaged for control sites and treatment sites.



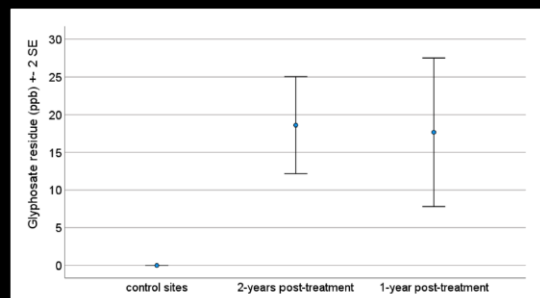
Composite samples were created to meet a minimum 5 g dry matter mass requirements for glyphosate residue analysis of each sample. At least three replicates were made from flowers across each site

Results: Glyphosate Residue

- Majority of composite floral samples 1- and 2-years post application contained residues.

- Mean level for 1-year post application: 19.6 ppb with 51.0 ppb being the highest value.

- Mean level in sample from 2-years post application): 18.9 ppb with 62.0 ppb being the highest value.



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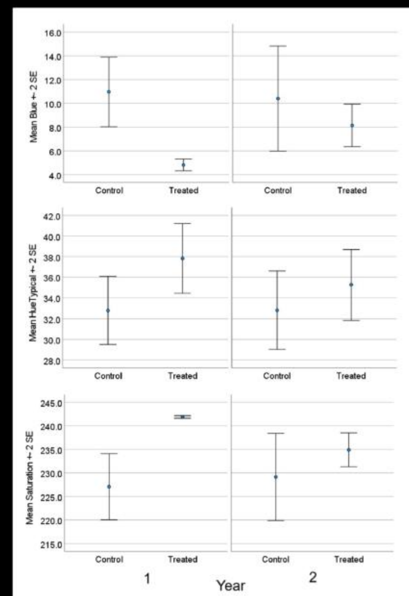
Results: Stamen Fluorescence

- No significant differences measured in colour characteristics between controls and 2-year post application sites.

- Significant differences in mean blue pixel intensity (mean blue), the most frequent hue observed (hue typical), and mean pixel saturation value (mean saturation) between control samples and 1-year post application sites.
  - Range of mean blue decreased, hue typical



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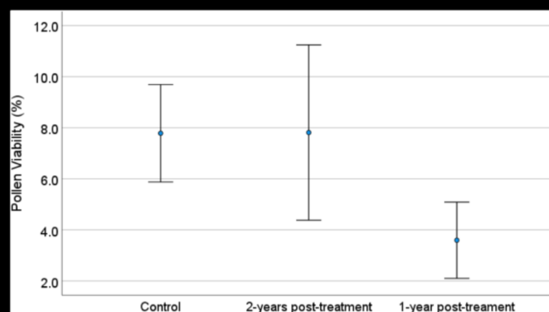
Fireweed stamen and pollen were substantially illuminated by the royal blue light fluorescence filter (wavelength 440-460 nm)

Results: Pollen Viability

- Significant differences measured between control and treated sites, between 1- and 2-years post application sites, and between 1-year post application and control sites.

- No significant difference observed between 2-years post application and control sites.



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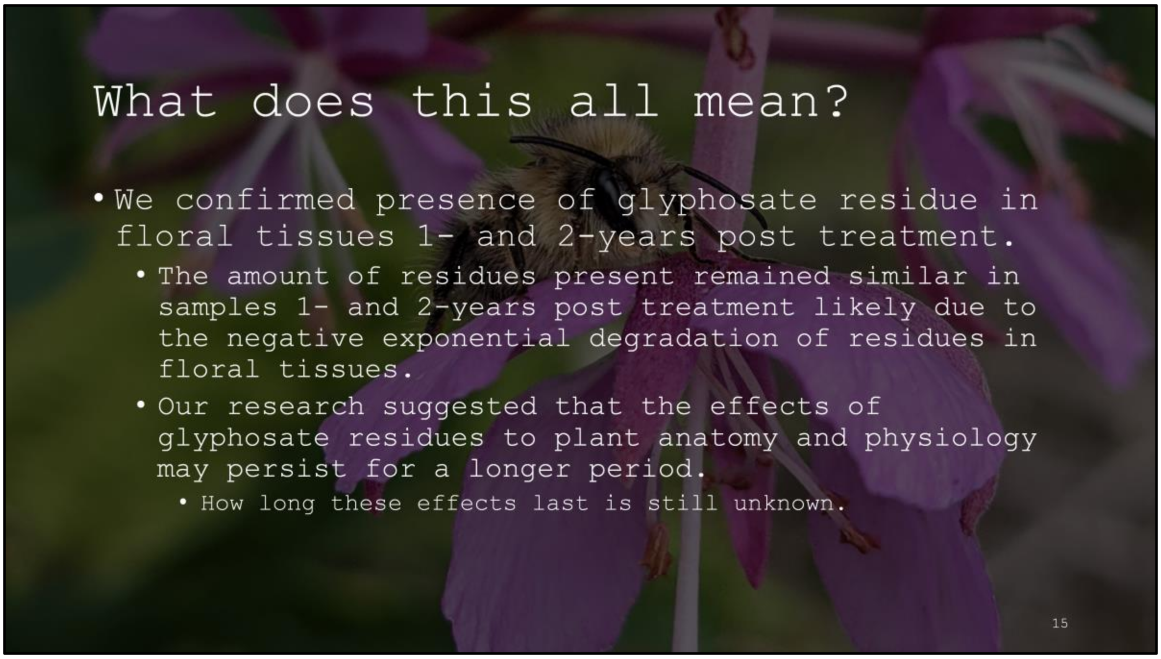
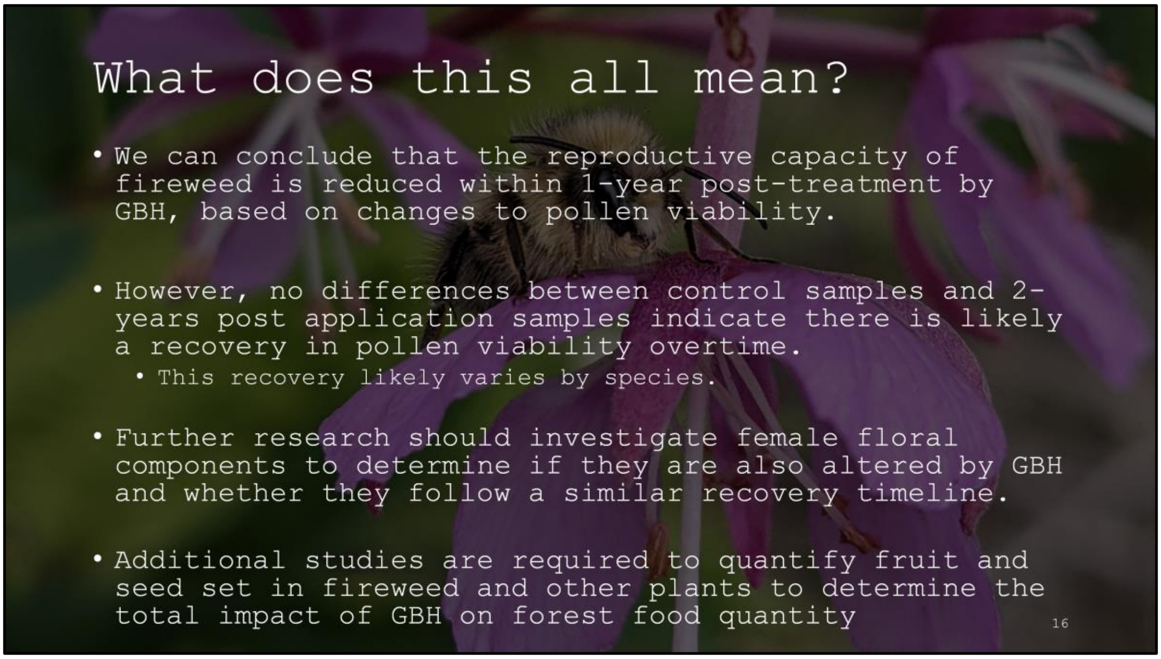
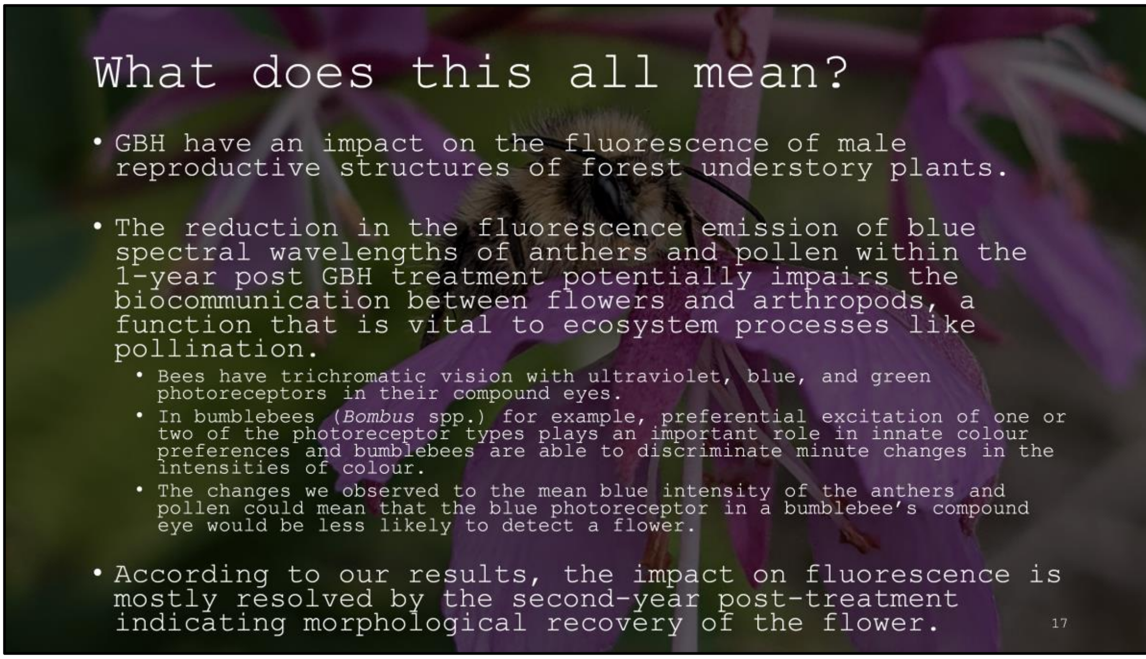


The amount of glyphosate residue present in floral tissues remained similar between one- and two-years post treatment, likely due to the negative exponential degradation of residues in floral tissues which leads to the majority of the glyphosate residue degrading within the first year after application.

Even though residues may not persist at high levels for greater than one year, our research suggests that the effects of glyphosate residues to plant anatomy and physiology may persist for a longer period; however, how long these effects last is still unknown.



Confirmed reduction in pollen viability in fireweed 1-year post application, indicating that GBH initially had a significant impact on the quantity of pollen that could successfully germinate and fertilize an ovule.



Additionally, the increase in typical hue observed indicates that the dominant wavelength present may no longer be the blue spectral wavelength. Combined with an increase in saturation, it is possible that the presentation of other spectral wavelengths in GBH treated plants are greater than that of the blue wavelength, potentially confusing biocommunication between flowers and pollinators.

Further research is required to determine if the changes in fluorescence we observed actually do result in changes in biocommunication.

Further research should also determine if the changes in fluorescence of fireweed flowers are correlated to changes in concentrations of anthocyanins, or other secondary metabolites, which serve other functions in addition to aiding in biocommunication.

Concluding Remarks

- Fireweed is a prominent component of the herbaceous layer in forests in northern BC.
- Fireweed shows stress symptoms after sub-lethal exposure to GBH used for vegetation management.
  - Includes reduced reproductive capacity (pollen viability) and reduced biocommunicative potential (stamen fluorescence).
- These changes have significant implications for the function of the ecosystem in these managed areas.
  - Including potential to change biocommunication with insect pollinators and the quality and/or the quantity of food produced for wildlife and humans.

Image by: A. Golt

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These changes to plant form and composition have significant implications for the function of the ecosystem in these managed areas, including potential to change biocommunication with insect pollinators, the quality, and/or the quantity of food produced for wildlife and humans.

We still do not have a good understanding of the impacts of GBH on forest understory plant reproduction and biocommunication with insect pollinators.

Thank you!

More research:
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Previous publication: https://doi.org/10.3389/fpls.2021.698202

Recent publication: .1038/s41598-025-16938-x

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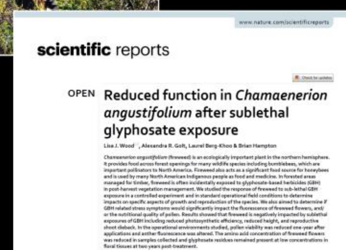

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OPEN Reduced function in *Chamaenerion angustifolium* after sublethal glyphosate exposure

Lisa J. Wood, Alexandra R. Golt, Laurel Berg-Khoo & Brian Hampton

Chamaenerion angustifolium (fireweed) is an ecologically important plant in the northern hemisphere. It provides food across forest openings for many wildlife species including bumblebees, which are important pollinators to North America. Fireweed also acts as a significant food source for honeybees and is used by many North American Indigenous people as food and medicine. In forested areas managed for timber, fireweed is often incidentally exposed to glyphosate-based herbicides (GBH) in post-harvest vegetation management. We studied the response of fireweed to sub-lethal GBH exposure in a controlled experiment and in standard operational field conditions to determine impacts on specific aspects of growth and reproduction of the species. We also aimed to determine if GBH related stress symptoms would significantly impact the fluorescence of fireweed flowers, and/or the nutritional quality of pollen. Results showed that fireweed is negatively impacted by sublethal exposures of GBH including reduced photosynthetic efficiency, reduced height, and reproductive shoot dieback. In the operational environments studied, pollen viability was reduced one-year after applications and anther fluorescence was altered. The amino acid concentration of fireweed flowers was reduced in samples collected and glyphosate residues remained present at low concentrations in floral tissues at two years post-treatment.