Investigation of Possible Biocontrol Resistance Within Select St. John's wort Populations in Southeastern British Columbia

Final Report

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BACKGROUND/CONTEXT

St. John's wort (*Hypericum perforatum*) is a perennial plant native to Europe that can reproduce and spread aggressively, particularly in disturbed sites and rangelands (ISCBC 2014). The first documentation of St. John's wort in the Southern Interior of British Columbia was in 1947 (Harris et al. 1969), and it has since become an invasive species throughout the region and across the Province (ISCBC 2014). St. John's wort is an excellent competitor; it diminishes native plant species, and can harm livestock in the process (Crompton et al. 1988).

Prior to the implementation of biocontrol, St. John's wort covered one million hectares of land in western North America (Crompton et al. 1988). A biocontrol program was implemented in British Columbia in 1951 (Crompton et al. 1988). In the years 1951-54, four species of *Chrysolina* spp. beetles were introduced in an attempt to eradicate the growing densities of St. John's wort (Harris et al. 1969). The *Chrysolina* spp. beetles are general defoliators and research suggests that three years of heavy feeding will kill a St. John's wort plant (Crompton et al. 1988). *Chrysolina hyperici* in particular is shown to be effective in controlling St. John's wort in the southern portion of BC; following treatment, less than 2% of the initial plant population remained (Harris et al. 1969). Furthermore, Harris et al. (1969) found that the beetle populations exhibited substantial growth, increasing 10-fold from an initial population of 3000 to 30,000 over a five-year period. The beetle was so successful that by the 1980s, releases were no longer required as distribution was extensive (MacRae, pers comm, Feb 3 2016).

Unfortunately, observations by Ministry of Forests, Lands and Natural Resource Operations and Rural Development (FLNRORD) indicate that St. John's wort is resurging on the landscape (MacRae, pers comm, Feb 3 2016, LaRade, pers comm Feb 6 2017) and the *Chrysolina* spp. beetles are not performing as well as they have historically. This could be due to the fact that St. John's wort has the ability to easily adapt to its surroundings, producing different plant growth forms under varying environmental conditions (Harris 1969). Stressors, such as herbivory, are known to trigger morphological and chemical predator defence mechanisms in plants (Karban & Myers 1989), such as the production of toxins (Cortesero et al. 1999). St. John's wort produces the toxic compounds hypericin in the dark coloured leaf glands (Zobayed et al. 2006), and hyperforin in the light coloured leaf glands (Soelberg et al. 2007). Ingestion of hypericin results in blisters, reduced reproductive performance, and in rare cases, death in livestock (Rockstein and Agosin 1978, Bourke et al. 2003). Hyperforin has been reported to serve as a defensive compound in response to herbivory (Beerhues 2006).

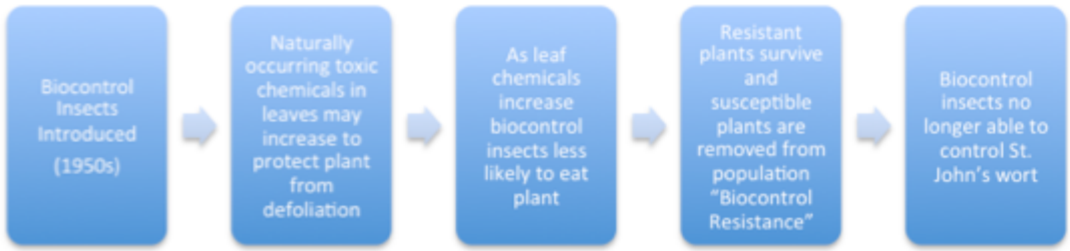

Figure 1 – Potential flow path for biocontrol resistance development in St. John's wort

St. John's wort may have the ability to overproduce hypericin and hyperforin as a predatory defense mechanism in response to the Chrysolina biocontrol agent. Previous fieldwork indicated that suspected resistant plants produced more light and dark coloured glands than susceptible plants (FIGURE 2); however the difference was not significant and we do not know if more glands translates into more hypericin/hyperforin. While suspected resistant plants had less defoliation than susceptible plants, the difference was not significant (FIGURE 3). Therefore, more samples are required.

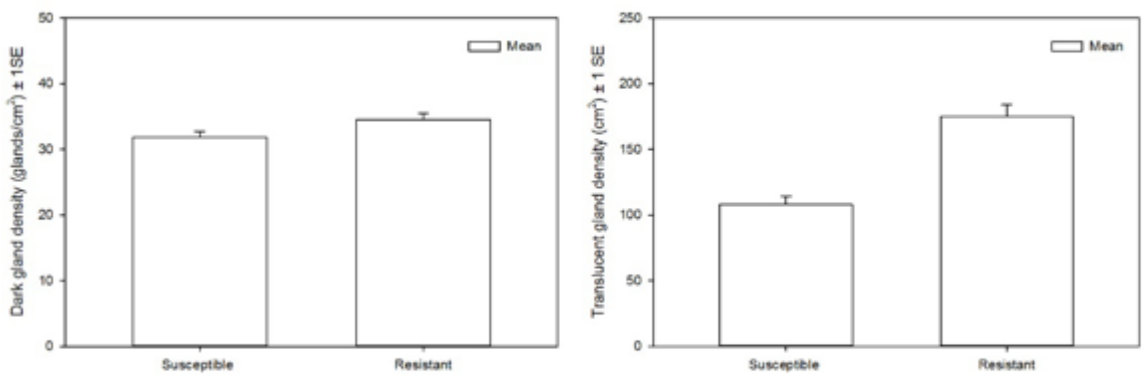

Figure 2 – Greater dark gland density (left image) and light gland density (right image) in suspected biocontrol resistant than susceptible St. John's wort plants.

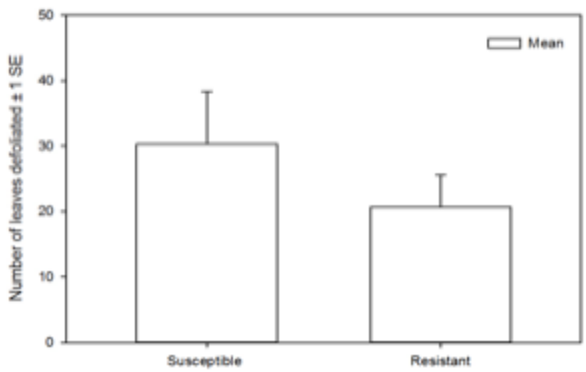

Figure 3 – Lower rates of defoliation in suspected biocontrol resistant than susceptible St. John's wort plants.

The results of the previous fieldwork indicate that there may be biocontrol resistance within the Columbia Basin region; however, further work is required to:

1. Verify resistance; determine levels of resistance; and how resistance might affect available forage.
2. Provide simple field based techniques for identifying potentially resistant plants; and, if needed, provide alternative tools for controlling resistant St. John's wort.

If resistance exists within St. John's wort, we need to reconsider our management of this species. Rather than waiting for the biocontrol insects to 'work', we need to be able to identify resistant populations and conduct research that will point to the best management options for control. If land managers choose to control resistant populations, they may prevent the introduction of resistance into new populations and ensure *Chrysolina* spp. continues to be an effective St. John's wort biocontrol agent.

OBJECTIVES:

Four objectives are housed under the umbrella of the project:

1. To determine if biocontrol resistance is present within St. John's wort populations at three sites within the Columbia Basin
2. To determine the effect of St. John's wort on available forage
3. To determine if there are morphological differences between resistant and non-resistant plants that can be identified in the field; and, if there are, develop a simple, field based tool to screen for potentially resistant plants
4. To present alternative chemical control options for St. John's wort

METHODS

Objective 1 – Determine if biocontrol resistance is present within St. John's wort populations within the Columbia Basin.

July 2 – 5th, 2018 three sites within the Columbia Basin were selected by Ministry of Environment and Climate Change Strategy - BC Parks (BC Parks) and Forest Lands and Natural Resource Operations and Rural Development (FLNRORD); a fourth site was also sampled at Marsh Creek in the Boundary Region (TABLE 1). The sites chosen by BC Parks and FLNRORD were sites where *Chrysolina* beetle had been observed in the past but was not longer present. The sites were Kikomun Creek Provincial Park (Kikomun), Cemetary Pasture, and WigWam (FIGURES 4-6). At each site, populations of St. John's wort were identified and plants were inspected for active feeding. If feeding was observed, then 10 'susceptible' and 10 'resistant' plants were harvested. If no feeding was observed then only 'resistant' plants were harvested. Susceptibility was determined by active foliar feeding, resistance was determined by abundant and active beetle activity on neighbouring plants yet no foliar feeding on the assumed resistant plant. All plants were transported to Thompson Rivers University (TRU).

Table 1 – Location of field sampling

Site	Population	Location	Easting	Northing
Kikomun	1	11U	627494	5456355
Kikomun	2	11U	628705	5457256
Kikomun	3	11U	627838	5455031
Kikomun	4	11U	628257	5454687
WigWam	1	11U	638773	5459157
WigWam	2	11U	639365	5457692
WigWam	3	11U	639644	5457920
Cemetary Hill	1	11U	629277	5457495
Cemetary Hill	2	11U	629265	5457390
Cemetary Hill	3	11U	629147	5457390
Marsh Creek	1	11U	462034	5442958

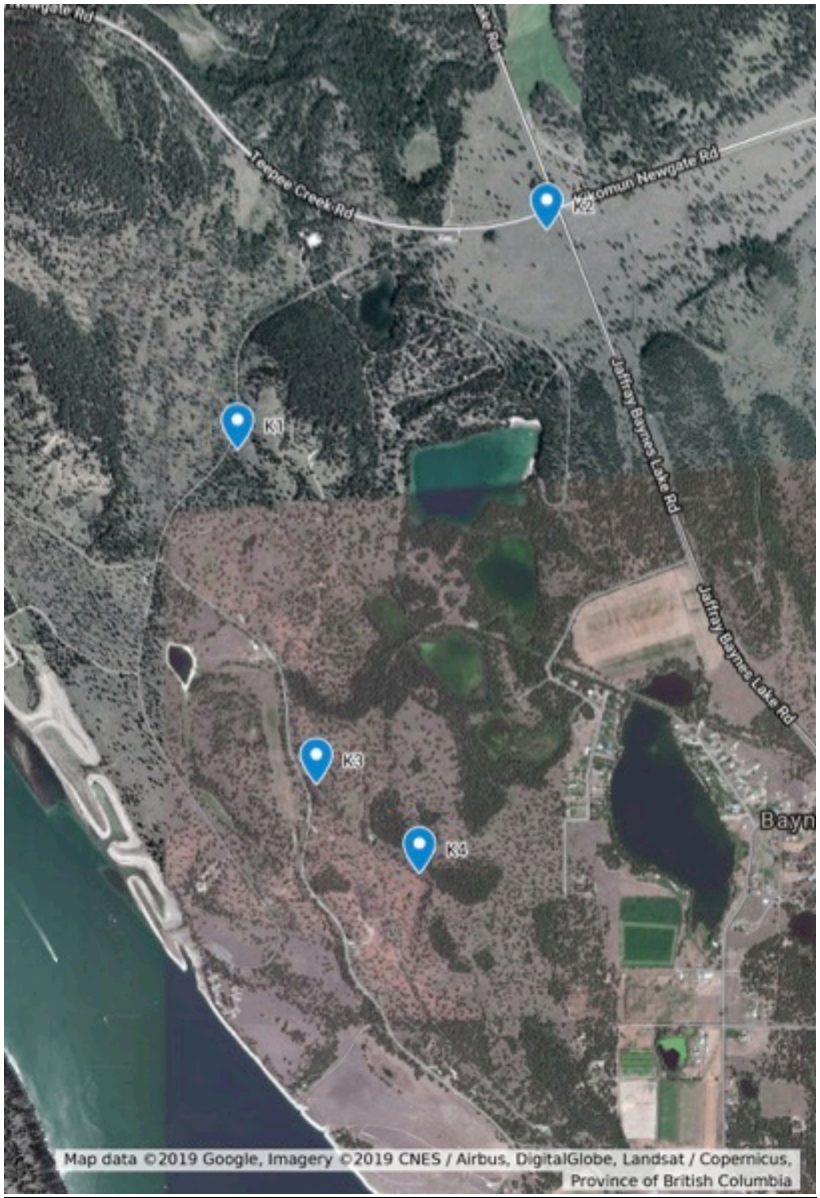

Figure 4 – Locations of four sampling sites within Kikomun Creek Provincial Park

Figure 5 – Locations of three sampling sites within the WigWam site

Figure 6 – Locations of three sampling sites within the Cemetary Hill Range Pasture

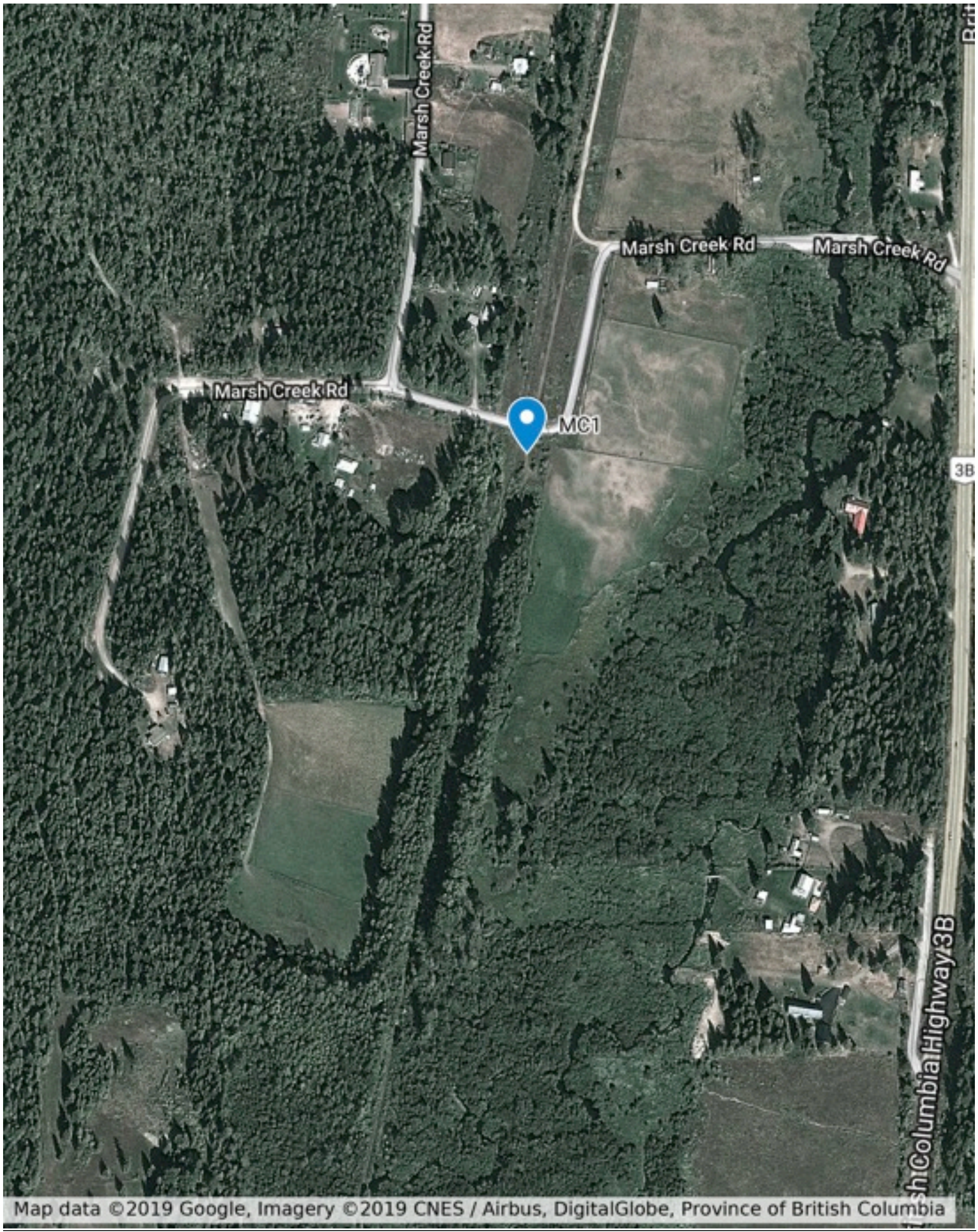

Figure 7 – Location of the sampling site at Marsh Creek

Gland Density

At TRU, 10 leaves from each plant were removed. All selected leaves were from the mid-section of the plant, fully expanded and of a representative size (i.e. – not disproportionately small or large). Each leaf was pressed between two microscope slides, to prevent leaf curl, and scanned at a 1400 d.p.i (FIGURE 7). The scanned images were imported into ImageJ to calculate leaf size and the number of light and dark glands. A ruler was included in all scans to ensure accurate leaf area calibration in ImageJ.

Figure 7 - Example of scanned leaves

Chemistry

Approximately 300 mg of leaf material was plucked from the same plant and/or plants from same population and placed into a plastic zipper bag and stored in a paper bag to prevent exposure to light. The leaf material was used to develop an HPLC method for the simultaneous determination of hyperforin, hypericin and pseudohypericin in leaf extract of St. John's wort. Since all three analytes are unstable in the presence of light, the sample solution was prepared under low light intensity by extracting the leaves with 2% dimethyl sulfoxide in methanol. The diluted or original extracted solutions were injected into a biphenyl column, and eluted with a mobile phase consisting of acetonitrile and 0.3% (v/v) orthophosphoric acid in water with a pH of 2.50 (80:20, v/v) at a flow rate of 1.5 mL/min. Hyperforin was detected at 273 nm with ultra-violet detector, simultaneously, hypericin and pseudohypericin were detected at 588 nm with photodiode array absorption detector. Hyperforin, hypericin and pseudohypericin had a linear relationship of peak area against concentrations over their concentration ranges, and their limits of detection and limits of quantification were 2.4605 and 8.2017 $\mu g \cdot mL^{-1}$, 1.4409 and 4.8029 $\mu g \cdot mL^{-1}$ and 1.7040 and 5.6799 $\mu g \cdot mL^{-1}$, respectively.

Feeding Experiment

Remaining plants from each population were used for a no-choice feeding experiment. Naïve beetles were collected from the Kamloops area and starved for 24 hrs before being placed on leaf samples. A susceptible or resistant shoot from each plant was placed in a small bottle of water (to prevent dessication) and then 2 beetles were placed on each shoot and allowed to feed for 1 hour. After feeding, each shoot was assessed for defoliation. Defoliation is presented as the percent of the leaf or leaves that was defoliated rather than as a percent of the total material available on the shoot. The beetles were monitored following the no-choice experiment to determine beetle survival.

Objective 2 - Determine the effect of St. John's wort on available forage

From July 5-10th, 2018, the three Columbia Basin sites (Kikomun, Cemetary Pasture and WigWam) were assessed for available forage within infested St. John's wort areas by MFLNRORD field staff using standardized range assessment tools. Within each plot, two $0.5m^2$ quadrats were used to sample available forage. Within each quadrat, plant material was harvested to 5 cm and separated out by St. John's wort versus 'Other'. The material was compared to Range Branch standards for a proper functioning ecosystem in Newgate and Baynes Lake area; typically 450-600 kg/ha.

Objective 3 – Identify simple, field based, characteristics to distinguish potentially resistant plants from susceptible.

During the field collection event, from July 2-5th, 2018, we identified potential field based characteristics for distinguishing resistant plants within a susceptible population. In the field, we assessed levels of defoliation and plant heights of the susceptible and resistant plants collected.

Objective 4 – Chemical control of St. John's wort

During the period from July 2-5th, 2018, at each population, one large plot was layed-out within the St. John's wort infestation. The plot was 50 by 100 ft in size. Within each plot, 5 clusters of 10 plants with no sign of *Chrysolina* feeding were identified as 'resistant' and permanently marked with a pin and flagging tape (FIGURE 8).

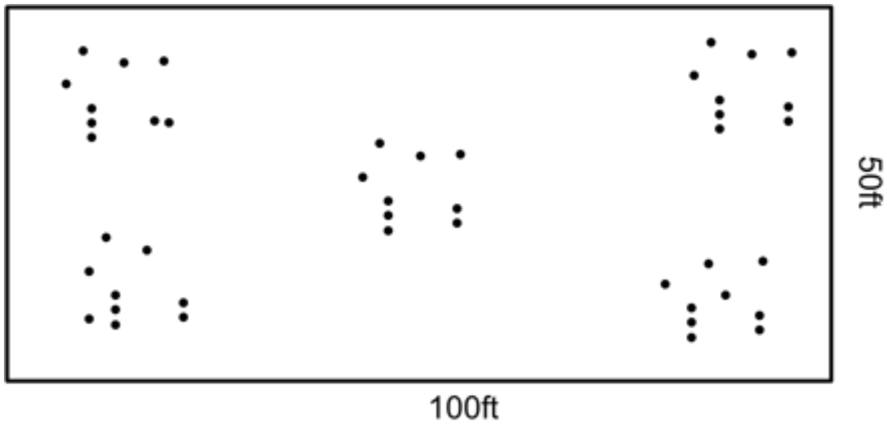

Figure 8 – Example of herbicide treatment plot lay-out. Each cluster of 10 dots represents 10 permanently marked St. John's wort plants. Each cluster was treated with a different herbicide, leaving 2 clusters as Controls.

On July 25 and 26th, three of the five clusters of 10 plants were treated with herbicide, the remaining two clusters served as Controls. Each of the three clusters was treated with a different herbicide. The herbicides used (and rates) were: Reclaim II A+B (200 g/ha + 1.7 l/ha), Milestone (0.5 l/ha), and Clearview (200 g/ha). All plots were visited October 15th and mortality was assessed.

RESULTS

Objective 1 – Determine if biocontrol resistance is present within St. John's wort populations within the Columbia Basin.

Unfortunately, a number of Kikomun and Cemetary Pasture sites had very little or no evidence of *Chrysolina* feeding; in fact, only 1 plant at Kikomun was found with evidence of feeding. However, the two locations were not statistically different from one another with regard to the resistant plants; thus, they were combined to increase the sample size.

Gland Density

Across all locations there were significant differences in both light and dark gland densities (FIGURE 9) between susceptible and potentially resistant plants.

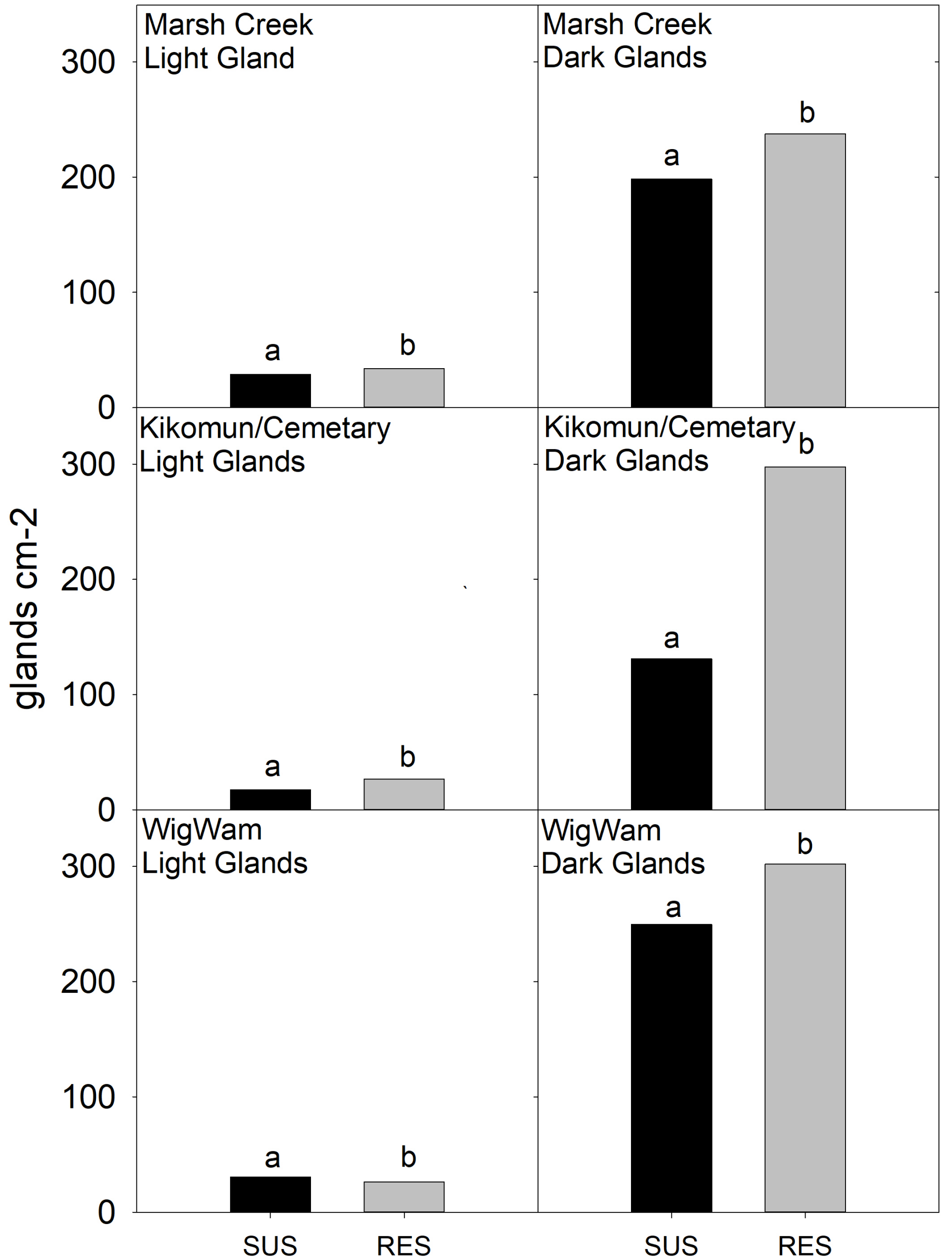

FIGURE 9 - Differences in light and dark gland density between potentially resistant versus susceptible St. John's wort plants at 3 locations: Marsh Creek, Kikomun/Cemetary Pasture, and WigWam. Bars with **a, b** above the measurements are significantly different ($\alpha = 0.05$)

Chemistry

While the leaf densities of dark and light glands were significantly different the abundance of chemicals in the leafs were not significantly different across all the sites and chemicals; an exception being hyperforin concentrations at Marsh Creek site (FIGURE 10)

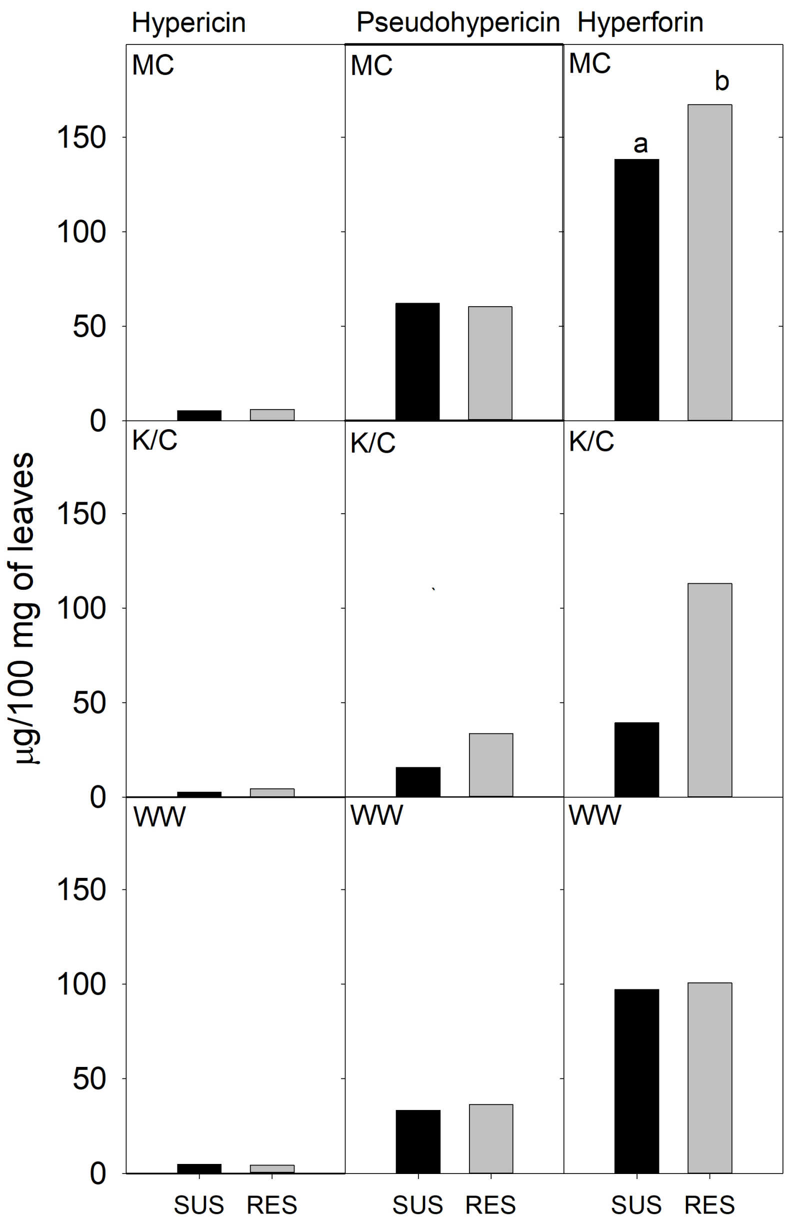

FIGURE 10 - Differences in chemical abundance between potentially resistant versus susceptible St. John's wort plants at 3 locations: Marsh Creek, Kikomun/Cemetary Pasture, and WigWam. Bars with **a, b** above the measurements are significantly different (α = 0.05)

Feeding Experiment

As with the chemistry results, the Marsh Creek plants were the only samples to have significant differences in defoliation rates (FIGURE 11).

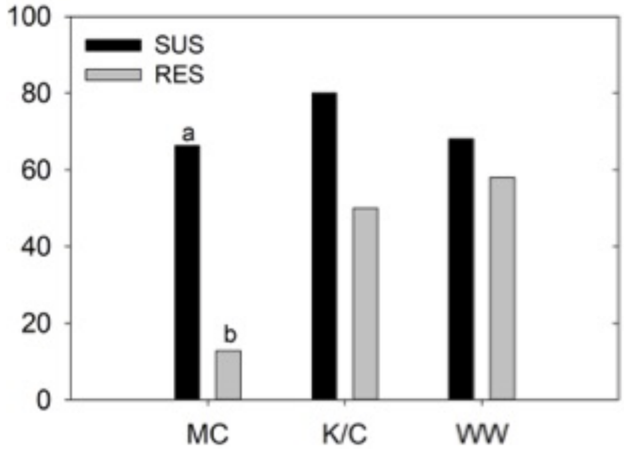

FIGURE 11 - Differences in defoliation rates between potentially resistant versus susceptible St. John's wort plants at 3 locations: Marsh Creek, Kikomun/Cemetary Pasture, and WigWam. Bars with **a, b** above the measurements are significantly different ($\alpha = 0.05$)

Objective 2 - Determine the effect of St. John's wort on available forage

At all sites, available forage was reduced by at least 50% (recall, expected forage values of 450-600 kg/ha). There was an inverse relationship between St. John's wort abundance and quality forage (FIGURE 12). Interestingly, the Kikomun sites are ungrazed and the Cemetary pasture sites are subject to grazing pressure.

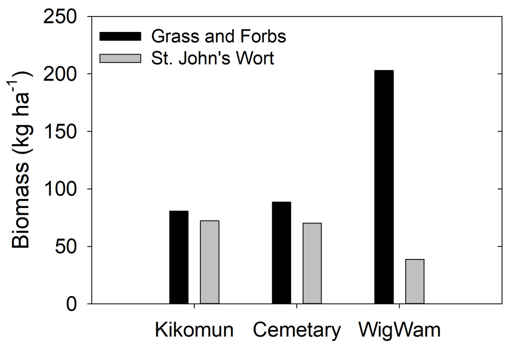

FIGURE 12 - Differences in average available forage production versus St. John's wort abundance at three St. John's wort infested locations: Kikomun, Cemetary Pasture, and WigWam.

Objective 3 – Identify simple, field based, characteristics to distinguish potentially resistant plants from susceptible.

Plant Heights

There existed no difference in plant heights at the Kikomun/Cemetary or WigWam sites between resistant and susceptible plants. However, at the Marsh Creek location there was a significant difference with the average heights of the resistant plants being 104 cm versus 78 cm for the susceptible plants (FIGURE 13). The difference in height was strikingly visible in the field.

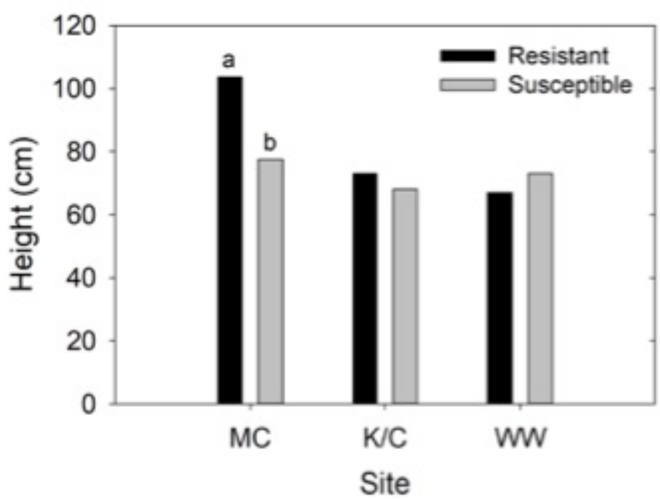

FIGURE 13 - Differences in heights between potentially resistant versus susceptible St. John's wort plants at 3 locations: Marsh Creek, Kikomun/Cemetary Pasture, and WigWam. Bars with **a, b** above the measurements are significantly different ($\alpha = 0.05$)

Leaf Area

Similarly, differences in leaf area were most noticeable at Marsh Creek; resistant plant leaf areas were nearly twice that of leaves from susceptible plants (FIGURE 14). Kikomun/Cemetary Pasture exhibited similar patterns with leaf areas being larger on resistant plants.

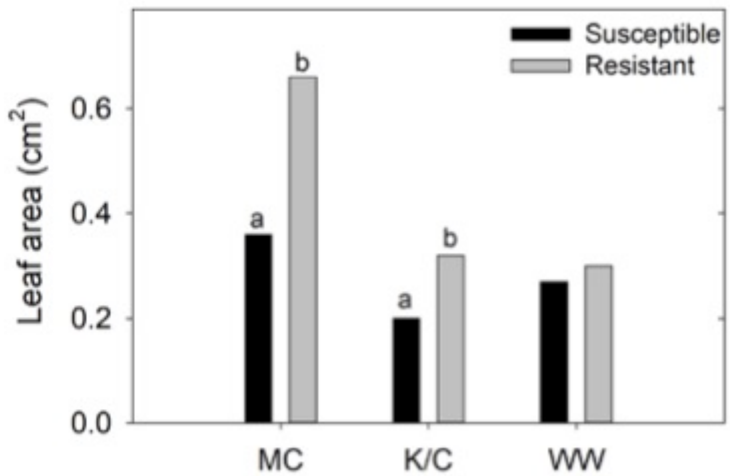

FIGURE 14 - Differences in leaf areas between potentially resistant versus susceptible St. John's wort plants at 3 locations: Marsh Creek, Kikomun/Cemetary Pasture, and WigWam. Bars with **a, b** above the measurements are significantly different ($\alpha = 0.05$)

Objective 4 – Chemical control of St. John's wort

Results from the herbicide trials indicate that Reclaim II provides the most consistent treatment effect across all three locations (FIGURE 15). While Milestone can result in high mortality, it also resulted in the lowest mortality at 38%. Clearview consistently underperformed across all locations. It is important to note that the application date of July 4-5 is very late for chemical treatment of St. John's wort.

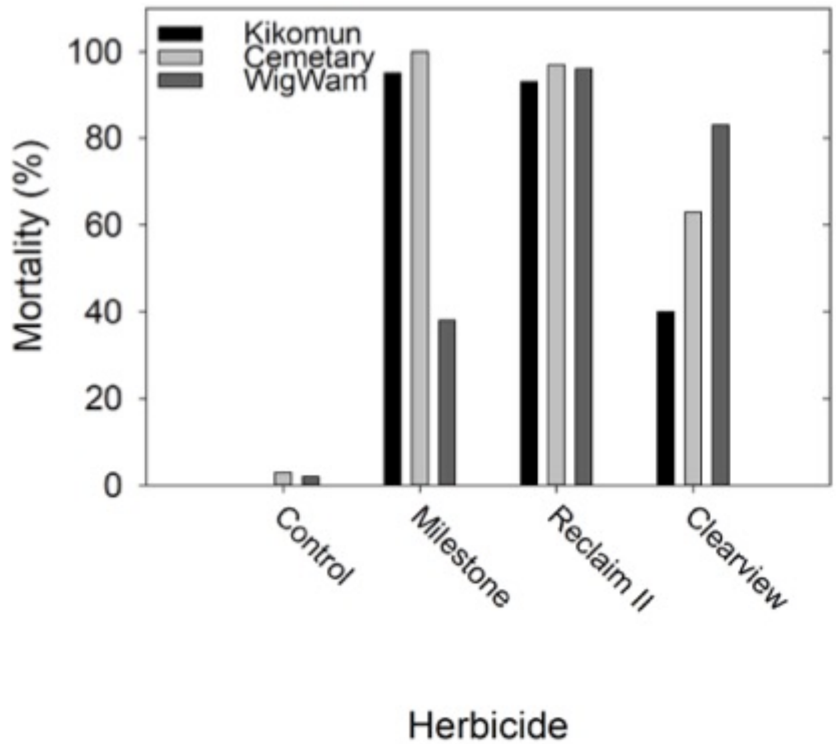

FIGURE 15 - Average differences in herbicide treatment effects at 3 locations: Kikomun, Cemetary Pasture, and WigWam for 3 herbicides: Milestone, Reclaim II, and Clearview versus a Control.

DISCUSSION and RECOMMENDATIONS

General Observations

The level of biocontrol presence and activity was very low across all locations. Because, there were no documented (IAPP) releases prior to 2005 in the Columbia Basin area and only 6 documented releases between 2005 and 2018, it is unclear if the low biocontrol abundance is due to resistance or under utilization of biocontrol in the area (FIGURE 16). It is likely that more releases were made between 1950-2005, but were undocumented and thus the information is unavailable. Therefore, sampling locations were based on verbal direction from government staff based on field observations.

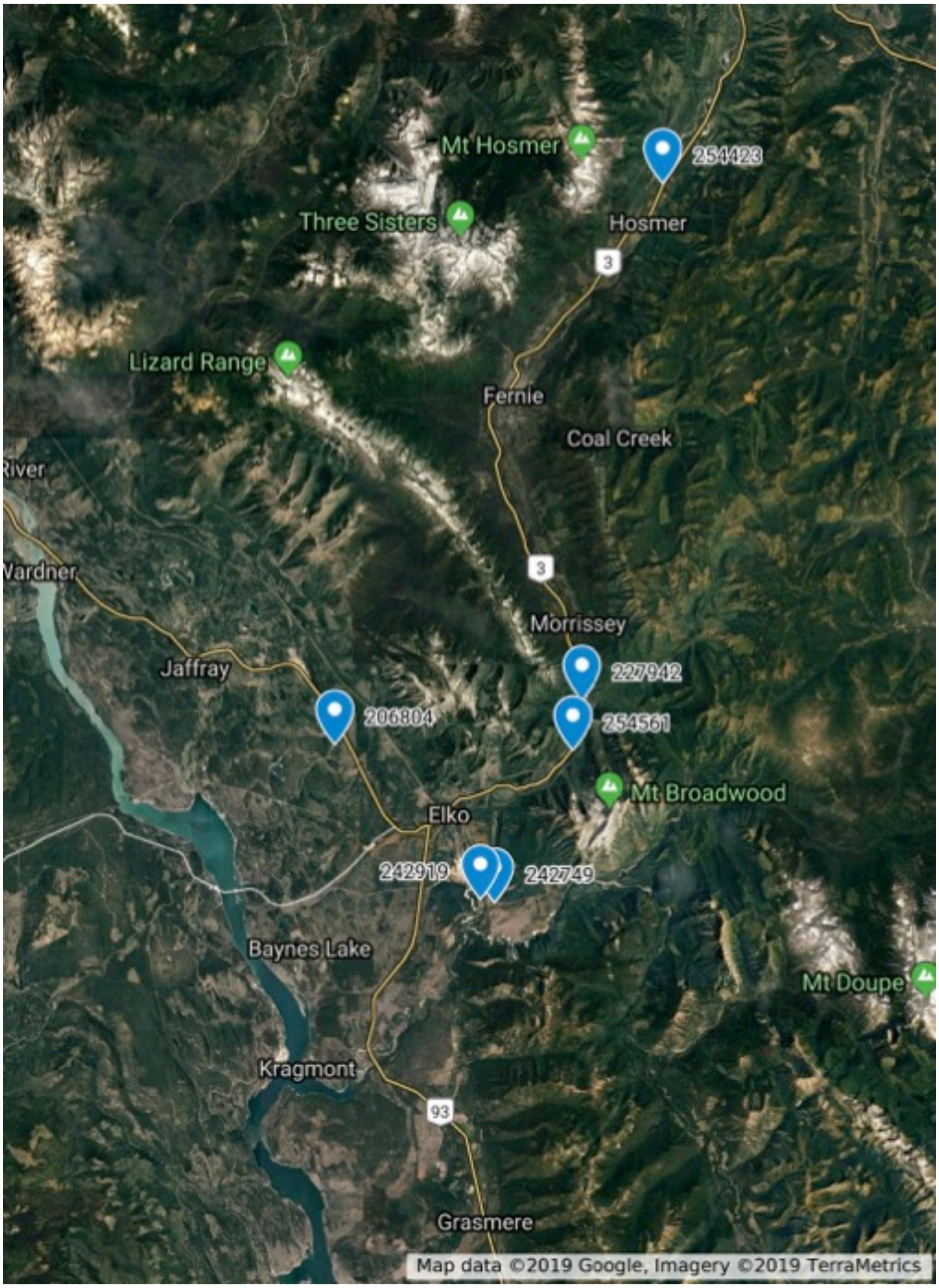

Figure 16 – Location of historical biocontrol releases (1950 – 2018). Data from IAPP (Invasive Alien Plant Program, Province of British Columbia)

Due to ecotypic variability there are large differences in measured values (leaf gland densities, chemical concentrations, leaf area, and heights) across locations, making it impossible to compare across locations; or generate values reflective of susceptible versus resistant. Therefore, it is necessary to compare susceptible and potentially resistant plants within a population to determine if a resistance exists. Fortunately the results from Marsh Creek provide some clear evidence for assessing biocontrol resistance in a population.

Field Trait for Potentially Biontrol Resistant St . John's wort

Outlined below are characteristics that, in combination, can be associated with potentially biocontrol resistant St. John's wort plants:

- Evidence of feeding on nearby plants but not feeding on potentially resistant plant, combined with
- Much greater height of plant, from 30-50% taller; combined with
- Leaves that are approximately twice the size of neighboring plants

Herbicide

Reclaim II is an excellent chemical control option for sites where increasing quality forage (grasses) is the primary land management objective. However, Reclaim II will remove broadleaves and woody shrubs; therefore, care must be taken when selecting sites for utilization if wildlife forage is a management priority.

Forage Availability

Ingestion of St. John's wort results in blisters, reduced reproductive performance, and in rare cases, death in livestock. Livestock susceptibility is such that horses are more susceptible than sheep, which are more susceptible than cattle, which are more susceptible than goats (horses>sheep>cattle>goats). Early work by Sampson and Parker (1930) conclude that overgrazing by cattle is one avenue that opens up a site to invasion. They also state that even low densities of St. John's wort can greatly reduce the grazing potential of an area if perennial grasses have been replaced by unpalatable winter annuals. The results from our study indicate that grazing pressure made no difference to abundance of St. John's wort or availability of forage (Kikomun 159 kg/ha versus Cemetary Pasture 153 kg/ha). It could be that the average percent cover of St. John's wort at Cemetary Pasture (41% at Kikomun versus 48% at Cemetary Pasture) is high enough to deter utilization of this area, such that the Cemetary Pasture could be considered non-use. Utilization and feeding behaviours of the cattle would need to be determined. As would the level of St. John's wort required to render an area non-useable.

Grazing Management Options

While there is no 'safe' time to graze St. John's wort, heavy grazing by goats is an excellent control measure, and goats tend to consume less than 20% of their diet in grass (Sampson and Parker 1930). Grazing management research by Arnott and Campbell in Australia (1994) began in 1979 on a heavily infested paddock of 90% St. John's wort. First, they aerial fertilized and seeded with *T. subterraneum* (subterranean clover). Livestock stocking rate was 8.2 dse/ha and was divided between 4.0 dse/ha goats and 4.2 dse/ha cattle. After 2 years the goat rates were progressively reduced to a stable rate of 2.2 dse/ha goats and 6.0 dse/ha cattle. By 1994 St. John's wort was reduced from 90% dense composition to 20% dense composition, 50% scattered St. John's wort, and 30% nil wort. The researchers do state that mixed herds likely work best in well-fenced areas where goat management is easier.

GENERAL CONCLUSIONS

The lack of biocontrol success and resurgence of St. John's wort on the landscape could be due to the underutilization of *Chrysolina* biocontrol in the region. While this insect is generally considered an excellent biocontrol agent, only six releases have been documented since 1950. Better utilization of the IAPP database is required in order to determine insect abundance and St. John's wort response on the landscape. Increasing the number of biocontrol agents in the region will give a better indication of whether or not biocontrol is actually a suitable control option, or if biocontrol resistance has indeed permeated the interaction between insect and St. John's wort, as is noted at Marsh Creek. In the mean time, following the tenants of Integrated Pest Management is critical. Integrated pest management (IPM) is a decision-making process for managing pests in an effective, economical and environmentally sound way (Province of BC). In order to practice IPM, land managers need to:

1. Planning and managing systems to **prevent** weeds – critical to this step is understanding the ecosystem and using Best Management Practices to maintain a healthy community and prevent weed invasion
2. **Identifying** pests – Not just identify, but understand the biology of the weed
3. **Monitoring** populations of pests – gathering info to determine best control option
4. Making control decisions based on potential damage, cost of control methods, value of production, impact on the environment (**Action threshold**)

5. Using **strategies** that may include a combination of biological, chemical, cultural and mechanical methods to reduce pest populations to acceptable levels
6. **Evaluating** the effects and efficacy of management decisions

An overall shift in our philosophy towards weed management is needed - there is no 'silver bullet'. Integrated Weed Management needs to be utilized in order to develop effective weed management. This means the synergistic use of biological, chemical, mechanical and cultural weed control methods to achieve effective results based on the biology of St. John's wort, the ecology of invaded ecosystems, and land management goals (FIGURE 17). In the below figure Cultural control options are highlighted as these options are ones that best incorporate understanding the biology of the weed *and* the ecology of the ecosystem. It is important to always prioritize land management goals before undertaking an IPM program. For example, while goat utilization may be appropriate for a number of sites and conditions, it is not suitable at WigWam because this area is prioritized for big horn sheep conservation and domestic goats can cause large-scale pneumonia die-offs in bighorn sheep (Teske Pers. Comm. 2019).

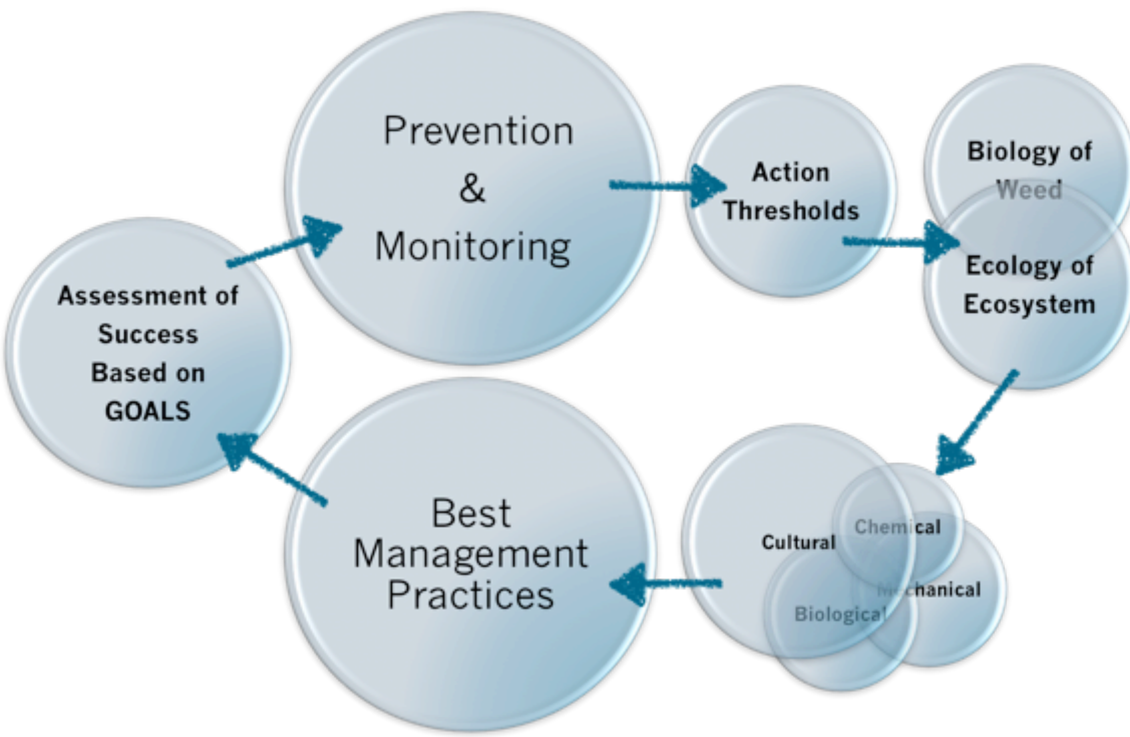

Figure 17 – Complete cycle of Integrated Pest Management (Tarasoff, unpublished)

Specific Conclusions Marsh Creek

There appears to be evidence of biocontrol resistance in the Marsh Creek population. The Marsh Creek site was the original *Chrysolina* spp. release location, with insects being released in 1951. This site has had over 60 years of insect pressure and thus is the most likely to have developed resistance. Marsh Creek was the only site with significantly different chemical compositions in the leaves of susceptible and potentially resistant plants with greater amounts of hyperforin. Hyperforin is produced in the light coloured leaf glands and has been reported as a defensive compound in response to herbivory (Beerhues 2006). Hyperforin has been documented to increase rapidly (within 24 hrs), although at variable rates, within defoliated plants in the greenhouse (Sirvent et al. 2003). The reduced defoliation rates documented in our study are likely a direct result of the higher levels of hyperforin. Other common features of resistance were also documented at Marsh Creek: resistant plants were taller with larger leaves, both characteristics that would result from reduced feeding pressure.

Possible development of biocontrol resistance has been noted in Australia for the mite *Aculus hyperici* (Jupp et al. in Jupp et al. 1997). Within just 6 years, plants exhibited some form of resistance whereby mite populations were unable to establish on some plants within populations. One of the challenges with St. John's wort is that individual plants are highly variable, even within a single population. However, high variability within St. John's wort biology, combined with possible resistance towards *Aculus hyperici* in Australia combined with our results indicates a high capacity for St. John's wort to develop resistance to biocontrol and other herbivory measures.

Recommendations for Marsh Creek

- Collect samples radiating out from Marsh Creek to determine the extent of possible resistance
- Train land managers on the field identifiers (taller height, larger leaf area, and absence of feeding when insects are abundant in the area) for potentially resistant plants within a population.
- This site has an abundance of native and desirable species mixed with St. John's wort; preservation of these species is critical; therefore, spot treat St. John's wort with Reclaim II, to remove the resistant population before resistance spreads to neighbouring populations. The native species should colonize the area once St. John's wort is removed.
- Monitor for success

Conclusions Kikomun Creek/Cemetary Pasture

Kikomun Site #2 did register as a 2016 release location in IAPP (Site 319429). At Kikomun Site #2 we found only two biocontrol agents on a single susceptible plant. The measured values of that plant were different than the potentially resistant plants; however, no conclusions can be made on a single plant. Given that the release was made only 2 years prior to the current research, it is likely that the insects are not well established rather than the population being highly resistant.

Recommendations for Kikomun Creek/ Cemetary Pasture

- Reintroduce biocontrol agents to each site (Kikomun 1-4 and Cemetary 1-3); allow the insects to establish over 2-3 years, and then resample susceptible and potentially resistant plants. Spray or goat browse the perimeter of the infestation to prevent weed spread while the biocontrol agents disperse
- Or, begin an aggressive herbicide (Reclaim II) or goat program to control St. John's wort and monitor native grass recovery. Consider reseeding sites if native plants do not respond to removal of St. John's wort. Within mixed stands where shrub and overstory canopy retention is desired, consider prioritizing goats for control.
- Continue to monitor chemical treatment effects
- If needed, reseed to desirable species
- Avoid disturbance of sites (over grazing or other) to prevent reestablishment of St. John's wort or other weedy species
- Monitor for success

Conclusions WigWam

WigWam was one of the few locations with active biocontrol present at all three locations. While the number of insects was still relatively low, susceptible plants were easy to identify. Observations from Government staff are that the biocontrol insects were robust at one time and St. John's wort was greatly reduced, but now insects are reduced and St. John's wort is resurging. These observations may be congruent with natural population fluctuations.

Recommendations for WigWam

- Because this site is managed for bighorn sheep, no domestic goat use is possible
- Release more biocontrol insects to recharge the population of insects. If possible, spot herbicide the perimeter of the St. John's wort infestation to prevent spread while biocontrol agents disperse.
- Continue monitoring for resistance development
- Continue testing herbicide efficacy to determine if there is a rate of Reclaim II that will remove St. John's wort without affecting the shrub community. Or, spot treat St. John's wort to avoid damaging bighorn sheep forage
- If needed, reseed site to desirable species once St. John's wort has been removed
- Avoid disturbance of area to prevent recolonization by St. John's wort or other weedy species

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