

The Impact of Primary and Secondary Robbing on Hummingbird Pollination

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Abstract:

Nectar robbing has been thought to lead to a reduction in plant fitness, although there is conflicting evidence for this assumption. Little evidence has been done on whether or not primary, or initial, robbing has a differing effect on plant fitness than secondary robbing does. To test the possible differences in robbing type, artificial nectar robbing was conducted on sixty *Ipomopsis aggregata* plants over the course of 6 weeks to examine the response of hummingbird pollinators to primary and secondary robbed plants. Stigmas taken from treated plants showed a significant decline in pollen receipt between un-robbed and secondary robbed treatments ($p = .0406$). Significant differences were not seen between un-robbed and primary robbing treatments, or between primary robbing and secondary robbing treatments. Based on prior research, the difference between the two robbing varieties and un-robbed plants is possibly due to increased avoidance of secondarily robbed plants. However, preliminary results do not support this claim.

Introduction:

Nectar robbing is a phenomenon that occurs when pollinating species obtain nectar through a punctured hole in the floral corolla, as opposed to the standard opening of the flower. In doing so, nectar robbers bypass the plant's reproductive organs, which has been shown to decrease fitness in some plant species (Irwin and Brody 1999, Traveset et al. 1998) by decreasing pollen deposits and subsequent seed set. Robbing has also been shown to alter the behavior of other pollinators toward the robbed plants (Irwin and Brody 1998, Zhang et al. 2014) by dissuading legitimate pollinators from visiting robbed plants. Despite the chronicled negative effects that robbing has on some plant species, other studies have found that robbing has a positive effect on plant fitness (Maloof and Inouye 2000), suggesting a complex interplay between nectar robbing and legitimate pollination. Nectar robbing is an activity that varies greatly by season and species (Irwin and Maloof 2002), but the high frequency of robbing on tubular corollas is because of morphological constraints, such as tongue length, that prevent bees and other robbers from accessing nectar the standard way (Inouye 1983).

Nectar robbing exists in two forms; primary robbing, in which an organism actively cuts a hole in the flower to obtain nectar, and secondary robbing, the use of an existing perforation to access nectar (Inouye 1980). Many species that are known to rob flowers do so secondarily because they lack the ability to puncture the flower on their own, and thus must resort to using holes previously made by primary robbers (Inouye 1983). Although the species that exhibit the two types of robbing differ, they are commonly considered to be of equal influence to plant fitness. Little research has been done to elucidate if differences in robbing type lead to differences in fitness on the afflicted plants. This experiment determined whether the two types of nectar robbing have differing impacts on plant fitness by using a hummingbird pollinated plant, *Ipomopsis aggregata*, and artificially controlling the robbing environments that the plants experienced.

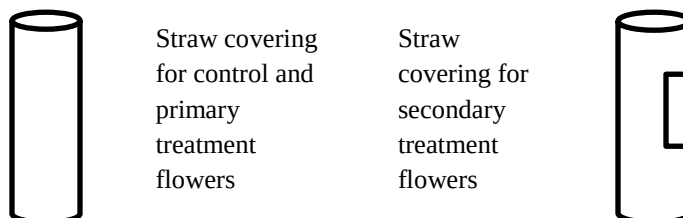
Previous research has shown that hummingbirds avoid robbed flowers of *I. aggregata* (Irwin and Brody 1998), although the cause for the avoidance was not determined. If hummingbirds react differently to different types of robbing, then there should be a difference in fitness between primary and secondary robbing. For example, if hummingbirds avoid one type of robbing more than the other, then the pollen receipt for the more frequently avoided plants will be lower than its counterpart, reducing the plant's overall fitness.

Methods:

The study was conducted near the Rocky Mountain Biological Laboratory (RMBL), in the Elk Mountains of Gunnison County, Colorado from July 7 – August 13, 2014 (Figure 6). At the RMBL, *I. aggregata* is mainly pollinated by two species of hummingbirds: broad-tailed hummingbirds (*Selasphorus platycercus*), and rufous hummingbirds (*Selasphorus rufus*). A third species of hummingbird, the calliope (*Stellula calliope*), was seen once during the course of the experiment. Both *Selasphorus* species are present near the RMBL late in the summer and are easily seen feeding from plants such as *I. aggregata*, a perennial with red tubular flowers adapted for pollination by hummingbirds. *I. aggregata* flowers for one year of its 2-7 year lifespan (Waser and Price 1989), which enables reproductive success to be measured during the course of one flowering season. The main primary robber in the area surrounding the RMBL is the bumblebee *Bombus occidentalis*, which frequently robs flowers of *I. aggregata*, and the main secondary robber in the system is *B. bifarius* (Pyke 1982).

For this experiment, 60 single-stalked *I. aggregata* plants were chosen for manipulation on a southwest facing hillside roughly 1 mile north of the RMBL, near the Avery Campground in the Gunnison National Forest. Plants with a single flowering stalk were chosen to cancel out the attraction that hummingbirds have toward large inflorescences of *I. aggregata* (Brody and Mitchell 1997). The plants were demarcated by a flag tied to the stalk before flowering and given a number 1-60, without repetition. Through the use of randomized assignments, 20 plants were not artificially robbed as the flowers opened (Control), 20 underwent a primary robbing treatment (Primary), and the remaining 20 underwent artificial secondary robbing (Secondary). Artificial robbing was done by cutting a small hole in the flower with scissors, and extracting the nectar by lightly squeezing the base of the flower and forcing any nectar inside into a 10µL microcapillary tube. The artificial robbing technique conducted effectively mimics the nectar robbing method done by *B. occidentalis* (Irwin and Brody 1998). Control plants were not artificially robbed, and to ensure that no natural robbing occurred, the tube of each flower was covered with a segment of a clear plastic drinking straw, which left the end of the flower open for pollination. Primary flowers were robbed once during the flower's lifespan and covered with the same time of straw protection as the control flowers, in order to prevent local insects from secondary robbing behavior. Secondary flowers were robbed multiple times during the flower's lifespan utilizing the hole made during the first artificial robbing bout. To enable natural secondary robbing yet keep the appearance of the test plants similar, secondary treatment flowers were covered in a drinking straw segment with a hole cut in the plastic. The hole in the straw was aligned with the hole in the flower so no additional robbing holes were naturally cut (Figure 1).

Figure 1:



Plants were treated five times weekly. Flower phenology was recorded to monitor when flowering was at its peak among the test plants. After treating new flowers, two hours of observation were done to record hummingbird visitations to test plants. Time of the visit, treatment plants visited, time spent at each plant, species of hummingbird, and the sex of the visiting hummingbird were recorded. Beginning on the second week of the experiment, open

stigmas were collected from each treatment plant; roughly 20% of available receptive stigmas were gathered each week. Pollen receipt on each collected stigma was counted after pressing stigmas onto microscope slides to measure the reproductive fitness of the plant's female components. Stigma slides were prepared using fuchsin, a gel that dyed pollen grains for easier counting. In the third week of the experiment, male fitness was tested by applying fluorescent powder to the anthers of new flowers on treatment plants (Irwin and Brody 1999). Half of the treatment plants were randomly assigned to be dye receptors, and the other half dye donors. Of the dye donors, each of the three treatments was randomly assigned a color of dye – blue, green, or pink. Two days after application of the dye, 20% of receptive stigmas were collected from the designated receptor plants, along with 50% of receptive stigmas on non-study plants. Dyed pollen was then counted on collected stigmas to test pollen dispersal of the different treatment types. The experiment was repeated one week later, reversing donor and recipient plants.

Analysis:

Pollen counts were totaled for each treated plant across the 5 week testing period and then averaged. The natural log of each plant's average was taken to create a more normalized data distribution for ANOVA. For hummingbird visitation, observed foraging bouts were analyzed by the mean time each bird spent per flower, and the proportion of flowers probed on visited plants. Male fitness was measured using a binary method; dyed flowers that donated pollen to a collected stigma were given a one, and those that did not were given a zero. Both visitation and male fitness were measured using ANOVA.

Results:

Treatment type had a significant impact on pollen receipt among the treated plants ($F = 3.2108$, $p = 0.0483$) (Figure 2). There was not a significant difference between pollen receipt of control and primary treatments ($p = .2756$), or between primary and secondary treatments ($p = .6644$), but there was a significant difference between the control and secondary treatments ($p = .0406$) (Table 1). Control plants had a significantly higher pollen receipt than secondary plants had.

For hummingbird visitation, analyses of treatment by mean visitation time per flower ($p = 0.1864$) (Figure 3), and treatment by proportion of flowers probed during visits ($p = 0.1891$) (Figure 4), were done. The results show that plant treatment does not have an influence on the time hummingbirds spend foraging on plants or the amount of plants probed during foraging bouts, despite the pollen receipt data that suggests a difference in hummingbird behavior toward the treatment types.

There was no significant difference in pollen dispersal among the treatments ($p = 0.4959$) (Figure 5). The data however followed a similar pattern to that of the observed hummingbird visitation.

Figure 2: Oneway Analysis of Pollen Logs by Treatment with Tukey-Kramer Test

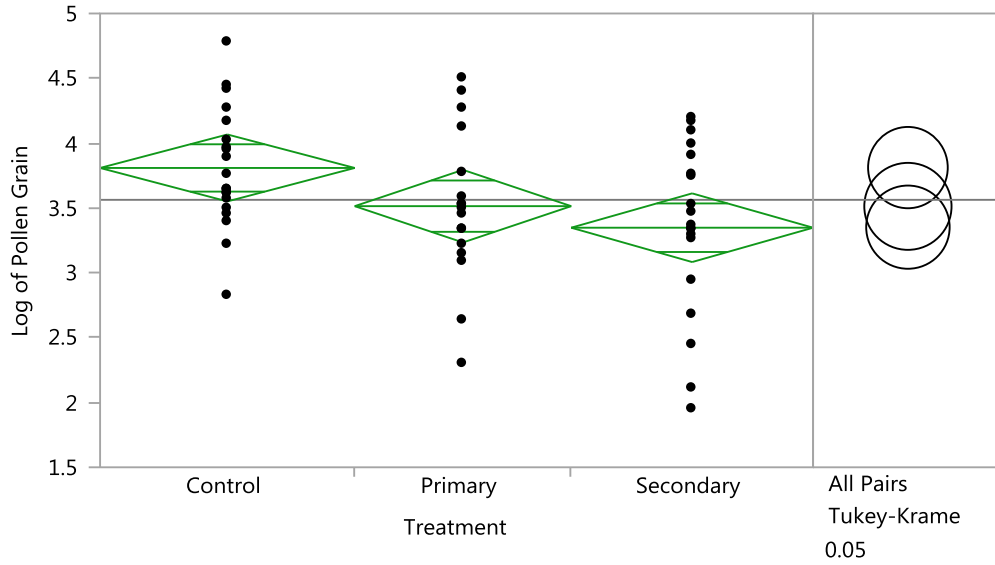
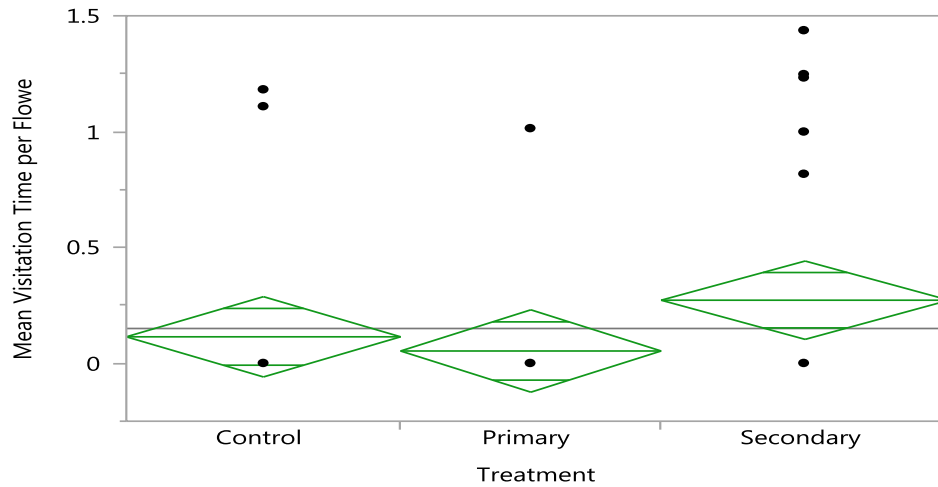


Table 1: Tukey-Kramer Analysis of ANOVA Analysis

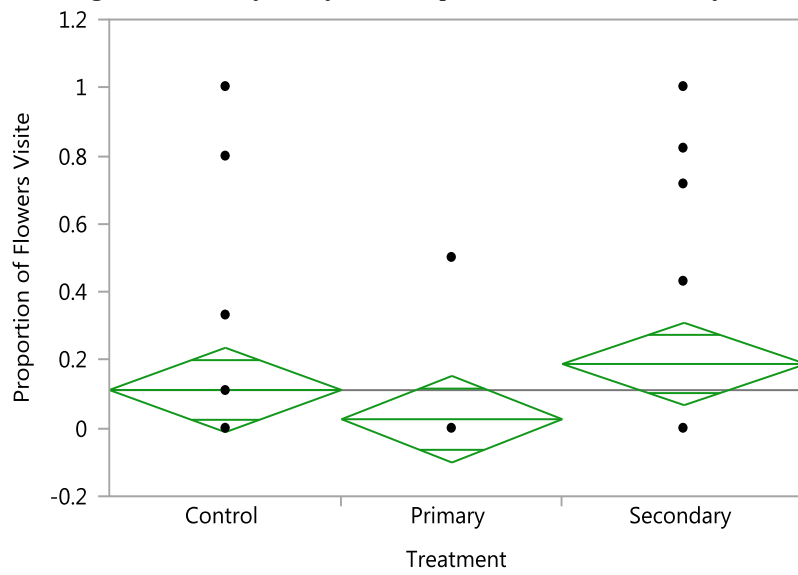
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
Control	Secondary	0.4607960	0.1843772	0.016213	0.9053790	0.0406*
Control	Primary	0.2945646	0.1898577	-0.163233	0.7523624	0.2756
Primary	Secondary	0.1662314	0.1921395	-0.297069	0.6295314	0.6644

Figure 3: Oneway Analysis of Treatment by Mean Visitation Time



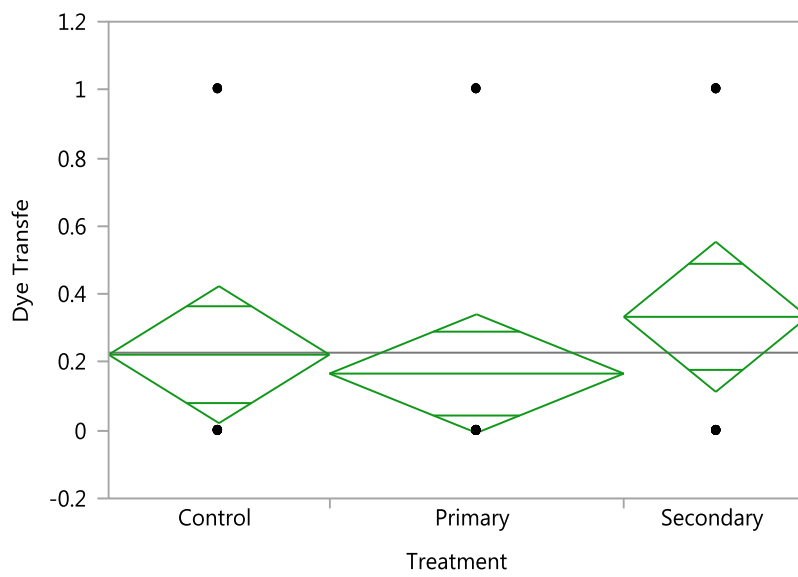
(F Ratio = 1.7301, p = 0.1864)

Figure 4: Oneway Analysis of Proportion Flowers Visited by Treatment



(F Ratio = 1.7152, p = 0.1891)

Figure 5: Oneway Analysis of Pollen Donation



(F Ratio = 0.7105, p = 0.4959)

Discussion:

The experimental results show that from a female fitness standpoint, the type of robbing that a plant is exposed to has a significant effect on its reproductive success. Although the data suggests decreased fitness for primarily robbed plants as compared to un-robbed plants, this decrease is not statistically significant. The difference between control and secondary robbed plants was significant, meaning that the seemingly minor distinction between primary and secondary has a major impact in the reproductive success of the plant. Based on previous research on the relationship between hummingbirds and robbed *I. aggregata* plants (Irwin and Brody 1998), it is possible that hummingbirds avoided secondarily robbed plants more often than primarily robbed ones. The more frequently avoided secondary treatment plants would have received less pollen as a result of the pollinator avoidance, possibly explaining the experimental results. Primarily robbed flowers did have lower pollen receipts, on average, than control flowers, which is expected based previous findings (Irwin 2000), but hummingbirds did not avoid this treatment as often as they avoided secondarily robbed plants. The experiment shows a difference in the impact that robbing has on male and female fitness. This difference has been observed before; reduction in female fitness due to robbing was observed by Irwin and Brody (1999), while studies have also shown that robbing can improve male fitness by promoting outcrossing and forcing birds to visit more flowers to meet their nectar requirements (Mayer et al. 2014). Although the pollen receipt data suggest avoidance, the observed visitation and male fitness data, although not significant, shows trends that run contrary to that suggestion.

On observed visits, a higher percentage of flowers were visited on secondary plants, and more time was spent foraging at each flower. *Ipomopsis aggregata* is a protandrous hermaphrodite, meaning that flowers originally exhibit male characteristics, then exhibit female traits later in its lifespan. In the context of optimal foraging theory, which postulates that animals seek to forage in a way that maximizes resource gains while minimizing resource losses (Pyke 1984), hummingbirds would be expected to prefer control plants, and the higher nectar volumes they provide, over secondary plants. It's possible that hummingbirds visited secondary flowers early in the flowers lifespan, but avoided them later after learning that the plants did not have as rich of nectar rewards. Early visitation of the flowers would promote the male fitness of the flowers, while later avoidance of the plants would hurt female fitness of the same flowers, possibly explaining the trends seen.

Low numbers of observed hummingbird visits are likely due to the relatively small number of test plants in the field as compared to the total number of *I. aggregata* in the vicinity. Sixty plants were treated in the field, and hummingbirds were frequently seen visiting plants near test plants. Increasing the test size would have likely yielded more observed visitations. Pollen receipt however serves as a suitable proxy for hummingbird visitation because pollen transfer is indicative of hummingbirds performing legitimate foraging on test plants. The lack of adequate data for male fitness is likely explained by monsoonal rainfall in the afternoons during the testing period. The dye used for testing was water-soluble, which likely meant that storms washed away some of the applied dye, thus lowering the amount of dye that was able to be transferred in the system

This experiment shows that primary robbing does not reduce plant fitness, while continued robbing of the same flower does. The tendency to pool primary and secondary robbing together and claim that they are equally detrimental to the affected plant is inaccurate. Secondary robbing, possibly because of the repetitive nature of the action and the damage that may

accumulate on the flower because of it, causes a significantly lower female fitness in *I. aggregata* than lack of robbing. Pollen receipt on primary robbed plants, although lower than that of control plants and higher than secondary robbed plants, was not significantly different than the other two treatments. From the perspective of the plant, getting robbed the first time will not significantly weaken its reproductive potential; it is after repeated robbing episodes only that the plant will see a significant drop in pollen receipt from its un-robbed state.

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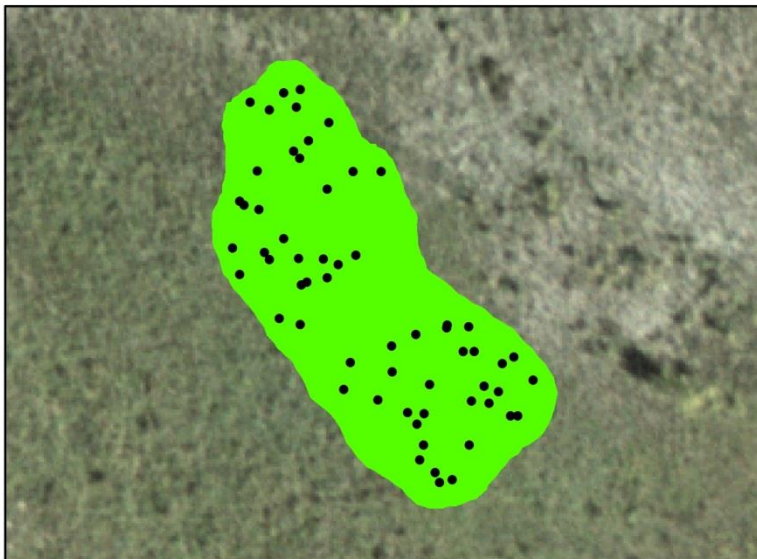
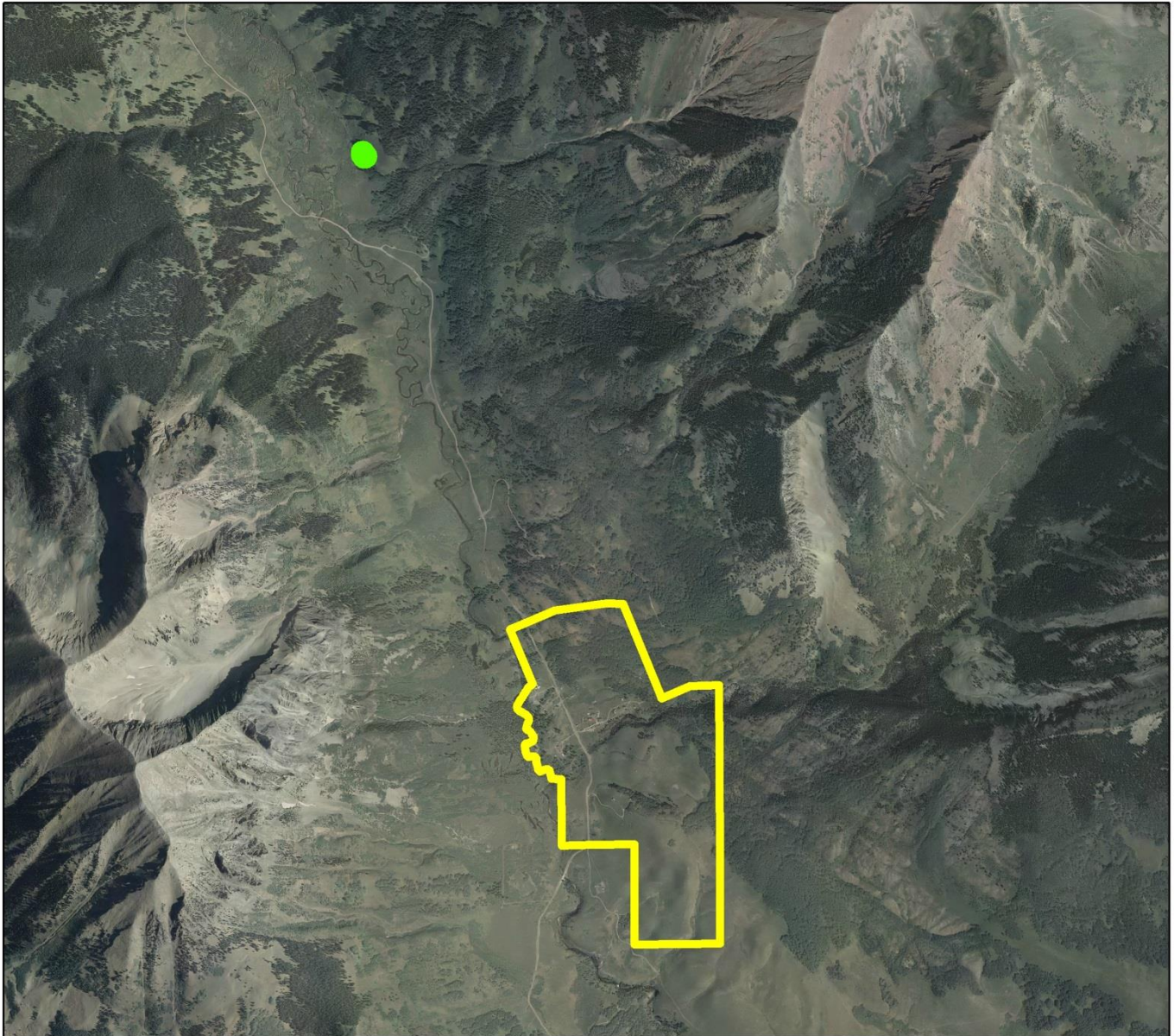


Figure 6: Study Site and Location in Gothic Valley

Above: Study Site (light green) in relation to the Rocky Mountain Biological Laboratory (yellow outline)

Left: Enlarged view of study area, with treatment plants shown (black circles)

0 0.15 0.3 0.6 0.9 1.2 Miles

