Factors affecting the infestation of the Canada thistle by the *Urophora cardui*

By

Olaniyi Ajala, Alec Zuhorski, and Bradley Swanson

Department of Biology

Central Michigan University

Mount Pleasant, MI, 48859.

.

ABSTRACT

The Canada thistle (*Cirsium arvense* [Scopolius] Asteraceae) is often considered the worst weed in North America. Once established in an area extirpation and removal is very difficult without extensive use of herbicides for more than 20 years to truly control. In contrast, biological control has been shown to successfully reduce Canada thistle reproduction in heavily infested areas. We investigated the ability of the fly *Urophora cardui* (Diptera: Tephritidae [Linnaeus]) to control Canada thistle at Pierce Cedar Creek Institute, highlighting the factors of attraction for the fly to the host plant. Our results show *U. cardui* is selective with respect to individual plants as the taller plants with a larger biomass and more seed heads were infected. *U. cardui* was also selective of the plant population size with 100% (8 of 8) of the large and (8 of 8) medium population sites compared to 12% (1 of 8) of the small population sites being infected. Additionally, the average seed head mass of infected plants (1.07 ± 1.25g) differed significantly ($t=4.25$, $d.f.=716$, $p<0.001$) from uninfected plants (2.07 ± 2.28g). This suggests that infection will reduce the reproductive capacity of the Canada thistle and will prevent spread to new locations. Though *U. cardui* will not affect the vegetative propagation of the Canada thistle, we conclude *U. cardui* are effective biological control agents with respect to reducing seed production in sufficient plant density.

INTRODUCTION

The break-up and drift of the supercontinent Pangea, in the early Mesozoic era (175 mya) resulted in geographic isolation which limited species from expanding their ranges into new areas (Raaymakers 2002; Lockwood et al 2007). Along with the limitation, species evolved independently within isolated areas allowing for natural patterns of biogeography (Raaymakers 2002; Queiroz 2005). However, humans have largely eliminated these barriers through advancements in global trade and transport, allowing species to invade new habitats (Raaymakers 2002; Queiroz 2005; Velde et al. 2006). Species ranging from micro-organisms to plants and vertebrates moved across the globe through the growing rates of human travel and trade (IUCN 2013). The consequences of these movements are the presence of increasing numbers of non-native species in these areas (Velde et al. 2006). Free from native predators, pathogens and competitors, non-native species often expand their introduced ranges through a wide range of vectors acting separately or together (Velde et al. 2006). Following expansion, non-native species negatively impact native species, environments, or ecosystem services (Lockwood et al 2007). Such non-native species are described as "invasive." Today invasive species are considered a main cause of plant and animal extinction and are a leading threat to food security, human and animal health (IUCN 2013; Pimentel et al. 2000). Invasive species have a significant impact on the global economy with damages and control efforts costing hundreds of billions of dollars each year (Pimentel et al. 2000).

Canada thistle (*Cirsium arvense* [Scopolius] Asteraceae) is a highly invasive plant in North America and is considered to be one of the worst weeds in the temperate regions of the world. Its native range includes Europe and North Africa, the eastern Mediterranean, southern Asia, Afghanistan, Iran, Pakistan, and as far east as China (Asadi et al. 2013). During the 1600s,

it was introduced to North America via crop seed. Since then, it has spread across the United States to 38 states, 25 of which have listed it as a noxious weed (Berner et al. 2013).

The life history of Canada thistle is ideal for rapid spread in novel habitats. It is a dioecious perennial with a wide tolerance for soil type and salinity levels, is capable of vegetative reproduction from the root system (producing ramet clusters up to 1.5 m in diameter), and is able to reproduce from seed (Cripps et al. 2011a). However, seed production and dispersal is not as extensive as in some invasive species as Canada thistle produces only 1000 – 1500 seeds per flowering shoot. The low level of seed production is offset by the 25-year longevity of the seed bank (Cripps et al. 2011a). Long-distance dispersal mainly occurs via animals or water and is common enough to produce the current distribution of Canada thistle across North America (Tiley 2010).

The distribution and spread of Canada thistle in North America is likely facilitated by absence of natural enemies (including parasites, predators and diseases). The enemy release hypothesis asserts that freedom from the pressures of natural enemies provides a competitive advantage to invasive plants by making favorable the allocation of less energy to the production of defenses and more to reproduction and growth (Carson 2014; Knochel and Seastedt 2009). Often, this diversion of energy allows invasive plants to become the dominant plant in invaded communities and pose a major threat to biodiversity in many habitats (Illinois Nature Preserve Commission 2004; Knochel and Seasted 2009). Cripps et al. (2011b) confirmed the bolstering effect of enemy release on Canada thistle; elimination of insect herbivory and pathogens found in its native range increased mean population growth and probability of development to reproductive maturity up to thirteen-fold in introduced areas.

One of the difficulties associated with controlling Canada thistle is that it commonly invades pastures and prairies. Canada thistle has the ability to grow from root fragments as small as 2 cm, thus the density of Canadian thistle is often increased in fields under cultivation as the majority of root pieces $\leq$ 20 cm deep in the soil resprout (Thomsen et al. 2013). This is also a problem with hand pulling of the plants as pieces of the root often break off in the soil allowing the plant to grow back the next season. Chemical agents are effective at controlling Canada thistle, but requires continued use for many years given the longevity of the seed bank and may not be feasible in areas where there is concern for the neighboring plants (Blasko and Nemeth 2006; Cripps et al. 2011a ; Samuel and Lym 2008). Mowing has also proven to be efficient at controlling the thistle if done following a rain (Bourdot et al 2011). Although this method might also be problematic depending upon the neighboring plants. Further, mowing reduces the cover of native species, and native species cover acts to decrease habitat resistance to invasion by Canada thistle (Larson et al. 2013). Biological control thus becomes a reasonable alternative for trying to minimize the presence of Canada thistle.

Over 75 species of insects have been found to feed on Canada thistle in its native range (Zwölfer 1965) and feeding trials found that six of these are monophagous on Canada thistle (Schroeder 1980). Several of these species have already been introduced to North America, either accidentally or as intentional biological controls agents. The leaf-feeding tortoise beetle (Coleoptera: Chrysomelidae *Cassida rubiginosa* [Müller]) is distributed throughout the eastern United States from Maine south to Virginia and west to South Dakota, and in Canada from Alberta east to New Brunswick (Ward and Pienkowski, 1978; Majka and Lesage 2008). The seed-feeding weevil (Coleoptera: Curculionidae *Larinus planus* [Fabricius]) is found from New York west to the Great Lakes and as far south as Maryland (Wheeler and Whitehead 1985).

However, the weevil has been found feeding on native thistle where it reduced population growth rate of the endangered Pitcher's thistle (*Cirsuim pitcheri* [Torrey] Asteraceae) by 10 – 12% and halved the expected time to extinction (Havens et al. 2012). The root-feeding weevil (Coleoptera: Curculionidae *Cleonis pigra* [Scopoli]) occurs throughout the Great Lakes region, throughout New York and southern Ontario, west to Michigan and east to eastern Quebec and New Brunswick (O'Brien and Wibmer, 1982; Anderson, 1987). Although Canada thistle and bull thistle (*Cirsium vulgare* [Tenore] Asteraceae), are the only known hosts in North America, *C. pigra* attacks a wide range of Asteraceae species in Europe (Anderson 1987), presenting risk for North American Asteraceae species. The phytopathogenic bacterium, *Pseudomonas syringae* pv. *tagetis* was found to produce up to 57% mortality in Canada thistle, but also damaged many Asteraceae species (Johnson et al. 1996) limiting its use in natural settings.

In contrast to the aforementioned species, *Urophora cardui* (Diptera: Tephritidae [Linnaeus]), one of the species suggested to be monophagous for Canada thistle, was not found to attack native thistles even in the absence of Canada thistle (Havens et al. 2012). This makes it a prime candidate for use in Michigan as a biological control agent where the endangered endemic Pitcher's thistle (*Cirsuim pitcheri* [Torrey] Asteraceae), and Hill's thistle (*Cirsuim hillii* [Canby] Asteraceae) are present and currently listed as a species of special concern (Freeland et al. 2010; Gautheir et al. 2010). *U. cardui* oviposits its eggs in the axillary buds of Canada thistle with hatching occurring in approximately one week. The development and overwintering of the larvae results in the formation of a stem gall up to 25 mm diameter and is followed by emergence in the early summer (Lalonde and Shorthouse 1985). The galls have a significant negative effect on the Canada thistle by serving as a resource sink, reducing turgor during periods of water stress, and limiting transfer through the xylem (Harris and Shorthouse 1996). A release of only

81 flies produced a 40% infestation rate across hundreds of Canada thistle individuals in southern Ontario with almost 100% seed failure in plants with galls (Laing 1977).

The goal of this research was to identify the factors which attract *U. cardui* to Canada thistle as well as the impact of the infection. We established a relationship between thistle population size and likelihood of population infection. Morphological characteristics potentially influencing infection of individual plants included height, weight, and number of seed heads. Seed head mass was used to show the detriment of infection on reproductive potential.

METHODOLOGY

Study Site

We conducted this research at the Pierce Cedar Creek Institute in Barry County, MI (42.536993, -85.302492), a 298 hectare property consisting of wetlands, forest, marshes, streams, lakes and prairies. Much of the property was in agricultural production until the early 1950s and is being converted to native prairies via herbicide applications, seeding and prescribed burns. These prairies are composed of native, non-native and invasive vegetation. Invasive plants such the Crown vetch (*Coronilla varia* [Lassen] Fabaceae), spotted knapweed (*Centaurea stoebe* [Lamarck] Asteraceae), bull thistle (*Cirsium vulgare* [Tenore] Asteraceae) and multiflora rose (*Rosa multiflora* [Thunberg] Rosaceae) are annually controlled both chemically and manually.

Population density estimates

Field work commenced May 2015 with a search of the property for locations with established Canada thistle populations. Our search efforts lasted until late June 2015 and were concentrated in prairies and fields where land managers knew Canada thistle was established. We searched each area in a serial manner by walking transects along the longest axis of each prairie or field separated by approximately 3 m. Upon the discovery of a Canada thistle patch, we mapped the perimeter with a L1 handheld GPS receiver (Mobile Mapper [Magellan Navigation Inc., Santa Clara, California]) and calculated the area of each patch using ArcGIS software (Esri; Redlands, California).

For large patches, we estimated population size by estimating density and calculating population size based on area of the patch; for smaller and sparser patches we took a census of the populations. We estimated density by locating the center of each patch based on the midpoint

of the longest transect and laying a $1m^2$ quadrat centermost. We then laid five additional $1m^2$ quadrats according to randomly generated angles ($0\text{-}360^o$) and distances (1-10 m) from the centermost quadrat. The number of Canada thistle ramets were averaged between the six quadrats to give a density estimate per m^2. For census measurements, we laid the minimal number of quadrats needed to include every individual in a patch. The total number of ramets in each patch was estimated as the product of each plant density estimate and patch area. We then classified the sites into small (<100 plants/site), medium ($100\leq$ plants/site <1000) or large ($\geq$1000 plants/site) population sizes. With a total of twelve sites of each population size, we selected eight sites for each population size in which to release *U. cardui*. Four sites from each population size were selected as control sites without *U. cardui* releases.

We purchased adult *Urophora cardui* from a private supplier (Biological Control of Weeds, Inc. MT, USA). Insect releases ranged from 20 to 200 individuals, depending on the thistle population size. Twenty insects were released into small populations, 40 insects into medium populations and 200 insects were released into large populations. All releases were made on the 26^{th} of June 2016.

Our harvesting efforts commenced on the 5^{th} of August and was completed by the 20^{th} of August 2015. We harvested the above ground biomass of infected plants (Canada thistle with at least one visible gall) by cutting plants at the base and storing plants in a polythene bag. Additionally, we harvested 50 uninfected plants (with no visible gall) from each release site and 30 uninfected plants from each control site. Harvested plants were allowed to air dry in a plant press. The variables recorded from each harvested plant include: plant height, plant mass, number of seed heads, mass of seed heads, number of galls, location of galls and mass of galls.

Statistical Analysis

We used a chi-square contingency test to determine the differences in percentage infection across the different population sites where insects were released (Fisher et al. 1999). For comparison of the plant mass, height, seed head mass and number of seed heads between infected and uninfected plants we used an unpaired two-tailed t-test (Elzinga et al. 2009). In addition, we ran a linear regression to determine if a relationship existed between seed head weight and gall mass (Woods et al 2008). The analysis were run on GraphPad Software (GraphPad Software, Inc; La Jolla, California).

RESULTS

In total, 1592 plants were harvested from 36 sites over an area of 6000m^2 (Figure 1; Table 1). Of these, 856 infected plants were harvested from release sites, 467 uninfected plants were harvested from the release sites and 269 were harvested from the control sites. We considered Canada thistle plants with swellings or outgrowths on the main stem or branching stem (galls) as infected and plants with no swellings or outgrowths as uninfected.

Infected plants were found in 70% (17 of 24) of the release sites. Within the release sites, we observed a significant difference in infection as 100% (8 of 8) of the high and (8 of 8) medium population sizes but only 12% (1 of 8) of the small populations were infected (x^2 =24, d.f.=2, p<0.001; Figure 2).

The infected plants (98.45 ± 34.99cm) differed significantly in height when compared to the uninfected plants (79.45 ± 36.07cm; t=11.26, d.f.=1588, p<0.001, Figure 3). Likewise, there was a significant difference in the number of seed heads of the infected (7.89 ± 13.29 seed heads/plants) and uninfected plants (6.29 ± 12.74 seed heads/plants; t=2.45, d.f.=1591, p=0.014, Figure 4). Similarly there was a significant difference in the plant mass of infected (17.15 ± 66.41g) and uninfected plants (11.57 ± 16.26g; t=2.26, d.f.=1588, p=0.024; Figure 5).

Galls on infected plants varied in both number (0.31 ± 0.68 galls/plant) and mass (14.41 ± 49.7g). The seed head mass of infected plants (0.08 ± 0.08g) differed significantly from the seed head mass of uninfected plants (0.24 ± 0.75g; t=4.25, d.f.=716, p<0.001, Figure 6). However, there was no significant relationship between gall mass and seed head mass (R^2=0.0006, p=0.59, Figure 7).

DISCUSSION

Our results show that *Urophora cardui* [Linnaeus] Astereceae are selective with respect to the plants and the populations of Canada thistle they infect. Specifically, they have a higher probability of infection in medium and large size populations and within those populations they prefer the taller plants with larger biomass and larger number of seed heads. Infection of the thistles resulted in reduced seed head mass independent of gall mass or number, indicating that the simple presence of a gall reduces reproductive potential of Canada thistle.

The population size of Canada thistle significantly influenced the likelihood that *U. cardui* would lay eggs at a site. Given that all of the high and medium population size sites but only 12% of the small population sized sites had any infected plants, our results suggest that there may be a minimum population size below which *U. cardui* will not oviposit. This disparity is unlikely just a result of the flies failing to encounter a Canada thistle because flies were always released next to Canada thistles, and the fly per plant ratio was greatest in the small population size sites. Avoidance may attributable to smaller thistle population not acting as windbreaks and retaining lower amounts of moisture over the winter for overwintering *U. cardui* (Turner et al 2009). Moisture levels retained by the plants in the winter are especially important for the emerging larvae as moisture levels are positively related to the ability to exit the gall in the spring (Turner et al 2009).

Within the breeding sites, *Urophora cardui* also appear to select individual thistle plants based on morphology. Our data suggest that plant height is a critical morphological character with *U. cardui* preferring taller plants. Taller thistle may likely be chosen because they have more oviposition sites. *U. cardui* oviposits onto axillary leaf buds (Lalonde and Shorthouse 1985), and as the shoot apical meristem grows, it leaves behind more axillary leaf buds

(Beveridge et al. 2003). Additionally, taller plants in groupings will provide canopies to protect and shelter insects from harsh weather and wind. Being small and fragile insects, *U. cardui* may easily lodge on the ground, especially with the dawning of unfavorable weather (Philip 2001; Turner et al 2009). *Urophora cardui* also selected plants with a greater biomass. A similar result showed that parasitoid abundance was significantly related to plant biomass as theseplants likely provide more resources to the developing larvae and more volume of space for use as habitat for adults (Haddad et al 2001). Lastly, we observed that infected plants had more seed heads. The floral odors of Canada thistle attract a wide range of insects including pollinators, herbivores and parasites (El-Sayed et al. 2008; Theis 2006). Hence *U. cardui* choose plants with more seed heads.

The presence of *Urophora cardui* galls significantly reduced the average mass of a seed head, which likely reduces the total number of seeds produced by these plants. Although the galls varied in number and mass, the variation did not influence seed head mass, suggesting that the presence of a gall alone will reduce the energy available for plant reproduction. This inference is in agreement with Laing (1977) who showed that thistles without galls produced twice as many seeds than those with galls. The reduced seed production of infected plants should decrease the likelihood of Canada thistle spreading to new areas which fulfills the objective of using a biological control agent (Wilson and Randall 2005). However, while the infection significantly affects sexual reproduction, we did not find any evidence of the impact of infection on asexual reproduction. This suggests that *U. cardui* is effective at controlling the spread of Canada thistle via seed dispersal, but is unlikely to be effective at extirpating local populations or even reducing current population size.

LITERATURE CITED

Anderson, R. S. 1987. Systematics, phylogeny and biogeography of New World weevils of the tribe Cleonini (Coleoptera: Curculionidae) Quaestiones Entomologicae 23: 431-709.

Asadi, G., R. Ghorbani, J. Karimi, A. Bagheri and H. Mueller-Schaerer. 2013. Host Impact and Specificity of Tortoise Beetle (*Cassida rubiginosa*) on Canada Thistle (*Cirsium arvense*) in Iran. Weed Technology 27: 405-411.

Berner, D., E. Smallwood, C. Cavin, A. Lagopodi, J. Kashefi, T. Kolomiets, L. Pankratova, Z. Mukhina, M. Cripps and G. Bourdot. 2013. Successful establishment of epiphytotics of *Puccinia punctiformis* for biological control of Cirsium arvense. Biological Control 67: 350-360.

Beveridge, C. A., J. I. Weller, S. R. Singer and J. M. I. Hofer. 2003. Axillary meristem development. Budding relationships between networks controlling flowering, branching, and photoperiod responsiveness. Plant Physiology 131: 927-934.

Blasko, D. and I. Nemeth. 2006. Efficiency and long-term effects of certain herbicides against Canada thistle (*Cirsium arvense* (L.) Scop.). Journal of Plant Disease and Protection 20: 739-745

Bourdot, G. W., G. A. Hurrell, R. A. Skipp, J. Monk, and D. J. Saville. 2011. Mowing during rainfall enhances the control of *Cirsium arvense*. Biocontrol Science and Technology 21: 1213-1223.

Carson, B.D. 2014. Phenology and dispersal of *Larinus minutus* Gyllenhal and *Larinus obtusus* Gyllenhal (Coleoptera: Curculionidae), two biological control agents of *Centaurea stoebe* ssp. micranthos (spotted knapweed) in Michigan. Biological Control 79:84-91.

Cripps, M. G., G. W. Bourdot, D. J. Saville, H. L. Hinz, S. V. Fowler, and G. R. Edwards. 2011a. Influence of insects and fungal pathogens on individual and population parameters of *Cirsium arvense*. Biological Invasions 13: 2739-2754.

Cripps, M. G., A., Gassmann, S. V. Fowler, G. W. Bourdot, A. S. McClay, and G. R. Edwards. 2011b. Classical biological control of *Cirsium arvense*: Lessons from the past. Biological Control 57: 165-174.

El-Sayed, A.M., J.A. Byers, L.M. Manning, A. Jürgens, V.J. Mitchell and D.M. Suckling. 2008. Floral scent of Canada thistle and its potential as a generic insect attractant. *Journal of Economic Entomology* 101:720-727.

Elzinga, C.L., D.W. Salzer, J.W. Milloughby and J.P. Gibbs 2001 *Measuring Plant and Animal Populations: A Handbook for Field Biologists.* John Wiley & Sons.

Freeland, J. R., J. Gillespie, C. Ciotir and M. E. Dorkena. 2010. Conservation genetics of Hill's thistle (*Cirsium hillii*) Botany 88: 1073-1080

Fisher, T.W., T.S. Bellows, L.E. Caitagirone, D.L. Dahlsten, C.B. Hufffaker and G. Gordh 1999 *Handbook of Biological Control: Principles and Applications of Biological Control.* Academic Press.

Gauthier, M., E. Crowe, L. Hawke, N. Emery, P. Wilson, and J. Freeland. 2010. Conservation genetics of Pitcher's thistle (*Cirsium pitcheri*), an endangered Great Lakes endemic. Botany 88: 250–257.

Haddad, N.M, D. Tilman, J. Haarstad, M. Ritchie and J.M.N. Knops. 2001. Contrasting Effects of Plant Richness and Composition on Insect Communities: A Field Experiment. The American Naturalist 158:17-35.

Harris, P., and J. D. Shorthouse. 1996. Effectiveness of gall inducers in weed biological control. Canadian Entomologist 128: 1021-1055.

Havens, K., C. L. Jolls, J. E. Marik, P. Vitt, A. K. McEachern, and D. Kind. 2012. Effects of a non-native biocontrol weevil, *Larinus planus*, and other emerging threats on populations of the federally threatened Pitcher's thistle, *Cirsium pitcher*. Biological Conservation 155: 202-211

IUCN. 2013. Invasive alien species: the urban dimension (Case studies on strengthening local action in Europe). http://data.iucn.org/dbtw-wpd/edocs/2013-027.pdf

Illinois Nature Preserves Commission. 2004. Spotted Knapweed (*Centaurea maculosa* Lam. Syn *C. biebersteinii* DC.). Vegetation Management Guidelines 1:1-6.

Johnson, D. R., D. L. Wyse, and K. J. Jones. 1996. Controlling weeds with phytopathogenic bacteria. Weed Technology 10: 621-624.

Knochel, D.G. and T.R. Seastedt 2009. Sustainable control of spotted knapweed (*Centaurea stoebe*). Management of Invasive Weeds 5:211-225.

Laing, J.E. 1977. Establishment of *Urophora cardui* L (Diptera, Tephritidae) in Southern Ontario. Proceedings of the Entomological Society of Ontario 108:2.

Lalonde, R. G. and J. D. Shorthouse. 1985. Growth and development of larvae and galls of *Urophora cardui* (Diptera, Tephritidae) on *Cirsium arvense* (Compositae). Oecologia 65: 161-165.

Larson, D. L., J. B. Bright, P. Drobney, J. L. Larson, N. Palaia, P. A. Rabie, S. Vacek, and D. Wells. 2013. Using prairie restoration to curtail invasion of Canada thistle: the importance of limiting similarity and seed mix richness. Biological Invasions 15: 2049 – 2063.

Lockwood, J.L., M.F. Hoopes and M.P. Marchetti. 2007. *Invasion Ecology.* Blackwell.

Majka, C. G. and L. Lesage. 2008. Introduced beetles of the Maritime Provinces, 7: *Cassida rubiginosa* Mueller and *Cassida flaveola* Thunberg (Coleoptera: Chrysomelidae). Zootaxa 1811: 37-56.

O'Brien, C. W. and G. J. Wibmer. 1982. Annotated checklist of the weevils (Curculionidae sensu lato) of North America, Central America, and the West Indies (Coleoptera: Curculionoidea). American Entomological Institute, Ann Arbor, Michigan, USA. 382 pp.

Philip, G.J. 2001. *Plant Strategies, Vegetation Processes, and Ecosysten Properties.*West Sussex, England: John Wiley & Sons Ltd.

Pimentel, D., S. McNair, J. Janecka, J. Wightman, C. Simmonds, C. O. Connell, E. Wong, L. Russel, J. Zern, T. Aquino and T. Tsomondo. 2000. Economic and environmental threats of alien plant, animal, and microbe invasions. Agriculture, Ecosystems and Environment 84:1-20.

Queiroz, A. 2005. The resurrection of oceanic dispersal in historical biogeography. TRENDS in Ecology and Evolution 20:68-73.

Raaymakers, S. 2002. The ballast water problem: Global ecological, Economic and Human health impacts. RESCO/IMO Joint Seminar on Tanker Ballast Water Management and Technologies Dubai, UAE: 22 pages.

Samuel, L. W., and R. G. Lym. 2008. Aminopyralid Effects on Canada Thistle (*Cirsium arvense*) and Native Plant Species. Invasive Plant Science and Management 1: 265-278.

Schroeder, D. 1980. The biological control of thistles. Biocontrol News and Information 1: 9-26.

Theis, N. 2006. Fragrance of Canada thistle (*Cirsium arvense*) attracts both floral herbivores and pollinators. *Journal of Chemical Ecology* 32:917-927.

Thomsen, M. G., L. O. Brandsaeter and H. Fykse. 2013. Regeneration of Canada Thistle (*Cirsium arvense*) from Intact Roots and Root Fragments at Different Soil Depths. Weed Science 61: 277-282.

Tiley, G. E. D. 2010. Biological Flora of the British Isles: *Cirsium arvense* (L.) Scop. Journal of Ecology 98: 938-983

Turner, S., S. Cesselli and M. Dewolf 2009 Operational Field Guide to the propagation and establishment of the biological control agent *Urophora cardui* (Canada thistle stem gall fly). British Columbia Ministry of Forests and Range

Velde, V.G., S. Rajagopal, M. K. Kollenaar, A. B. D. Vaate, D. W. Thieltges and H. J. MacIssac. 2006. Biological invasions: concepts to understand and predict a global threat. Ecological Studies 191:61-90.

Ward, R. H. and R. L. Pienkowski. 1978. Biology of *Cassida rubiginosa*, a thistle-feeding shield beetle. Annals of the Entomological Society of America 71: 585-591.

Wheeler, A. G., Jr. and D. R. Whitehead. 1985. *Larinus planus* (F.) in North America (Coleoptera: Curculionidae: Cleoninae) and comments on biological control of Canada thistle. Proceedings of the Entomological Society of Washington 87: 751-758.

Woods, D.M., M.J. Pitcairn, D.B. Joley and C.E. Turner. 2008 Seasonal phenology and impact of *Urophora sirunaseva* on yellow star thistle seed production in California 47:172-179.

Wilson, M.L. and C.R. Randall. 2005. Biology and Biological Control of Knapweed. *U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team*: 89 pages.

Zwölfer, H. 1965. Preliminary list of phytophagous insects attacking wild Cynareae (Compositae) in Europe. Technical Bulletin Commonwealth Institute of Biological Control 6:

81-154.

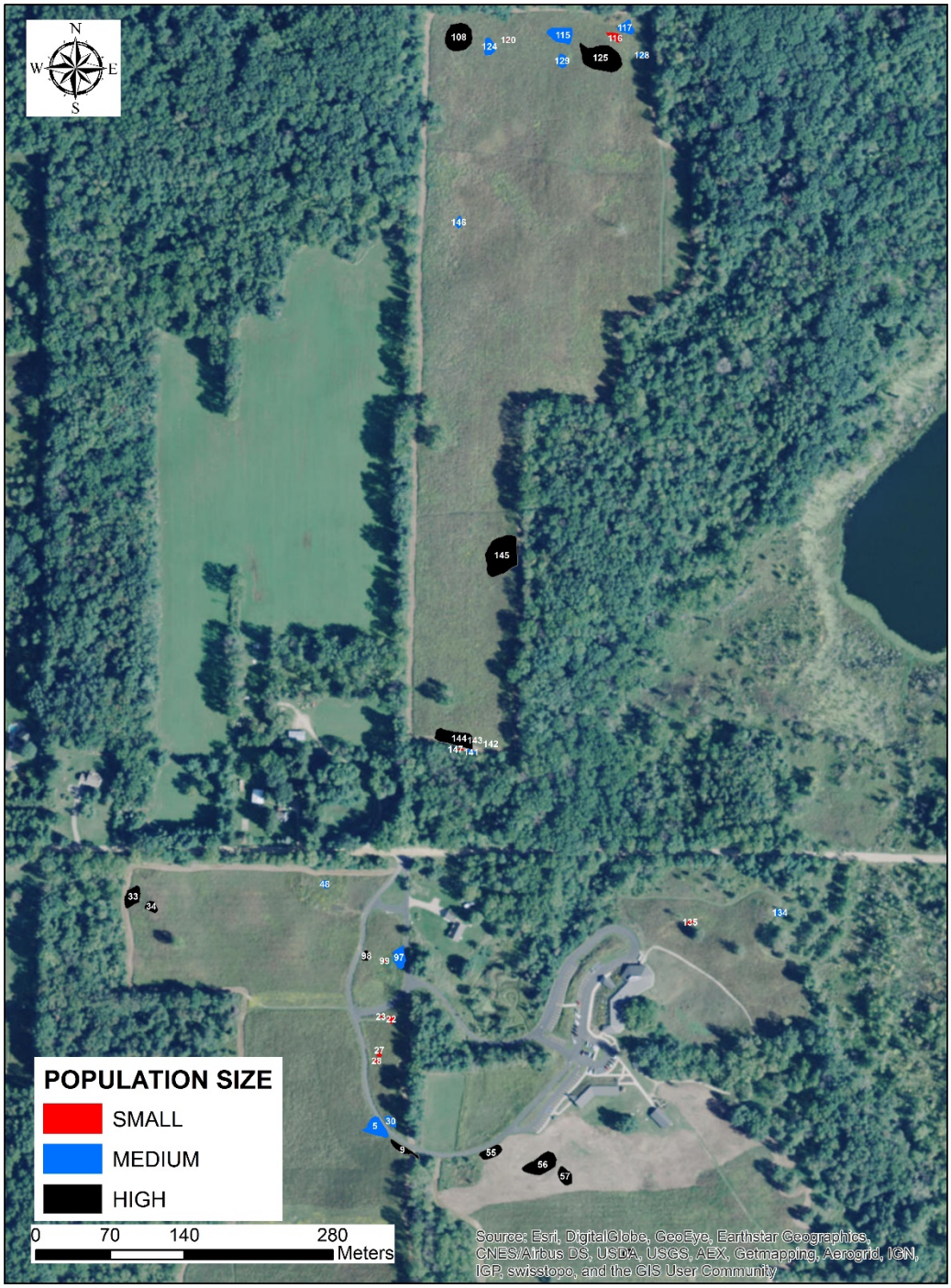

Figure 1: Study sites on the Pierce Cedar Creek Institute property showing the population sizes of the Canada thistle

Table 1: Area and Total number of plants of the different Canada thistle population sizes in the study site.

POPULATION SIZE	SITE ID	UNIT	AREA (m^2)	TOTAL NUMBER OF PLANTS
Small	120	Experimental	1	1
	142	Control	1	1
	25	Experimental	1	2
	99	Experimental	3	5
	143	Control	1	7
	23	Experimental	3	9
	27	Experimental	3	9
	135	Control	4	17
	28	Experimental	6	25
	147	Control	5	29
	22	Experimental	32	53
	116	Experimental	73	98
Medium	146	Control	46	115
	128	Experimental	30	204
	134	Control	49	251
	141	Control	20	270
	129	Experimental	93	542
	124	Experimental	130	584
	30	Experimental	66	594
	117	Experimental	126	610
	97	Experimental	174	637
	115	Experimental	274	640
	5	Experimental	253	802
	48	Control	45	965
High	34	Control	106	1205
	69	Experimental	100	1230
	98	Experimental	57	1271
	108	Experimental	572	2191
	144	Control	462	3160
	145	Control	951	3171
	125	Experimental	803	3344
	9	Experimental	149	3375
	33	Control	240	5990
	55	Experimental	190	7584
	57	Experimental	176	8082
	56	Experimental	446	10843

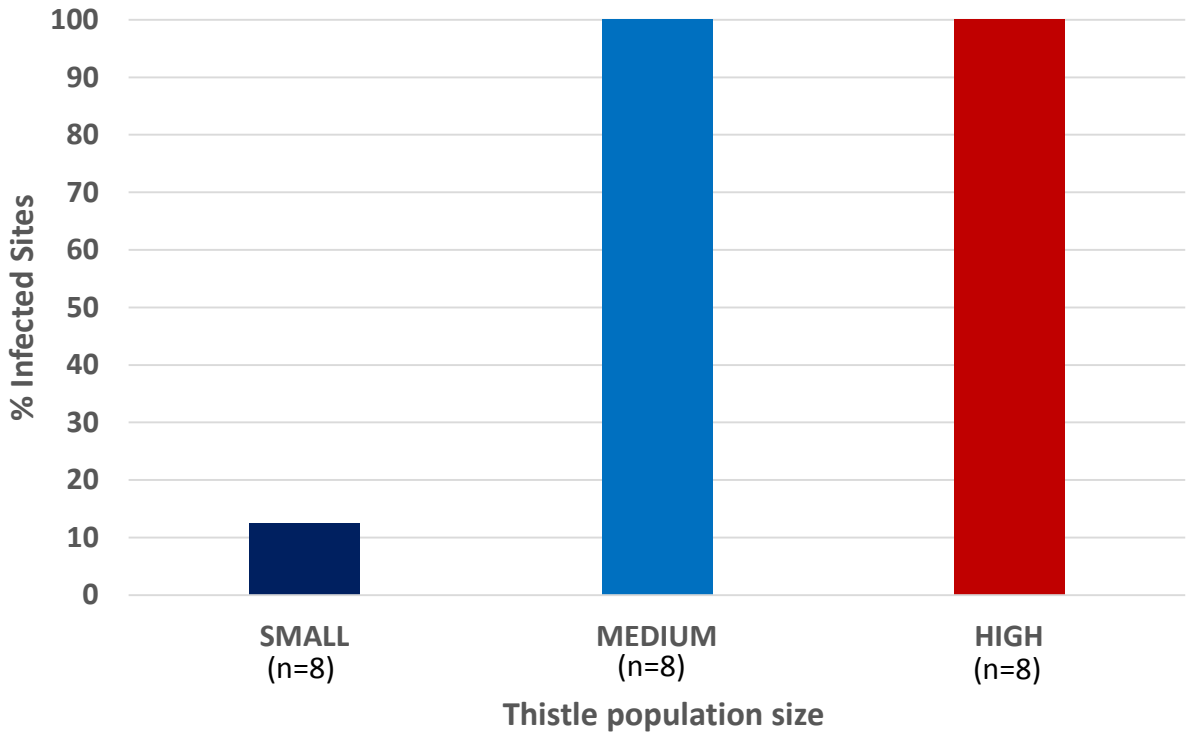

Figure 2: Percentage infection in small, medium and high population sizes in experimental areas. (x^2= 24, d.f. = 2 , p<0.00001)

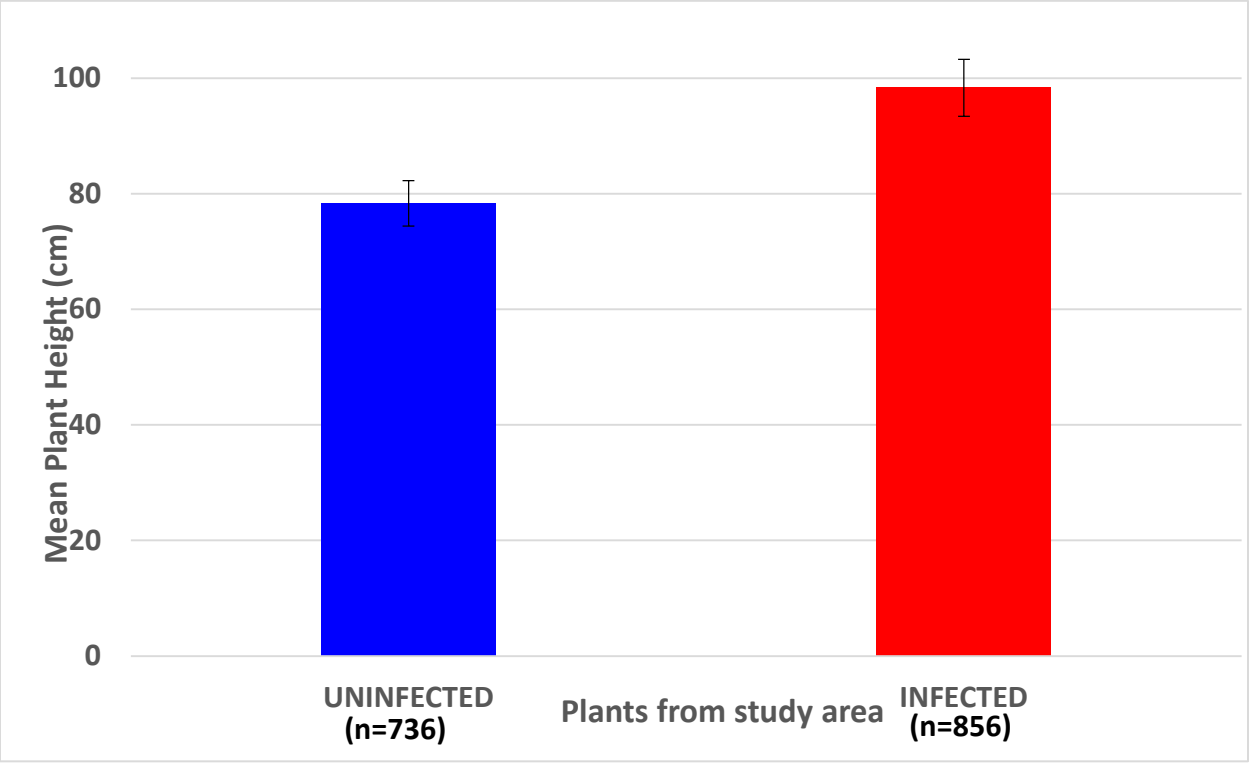

Figure 3: Average plant height of infected and uninfected plants from the study area ($t = 11.2601$, $d.f = 1588$, $p<0.0001$)

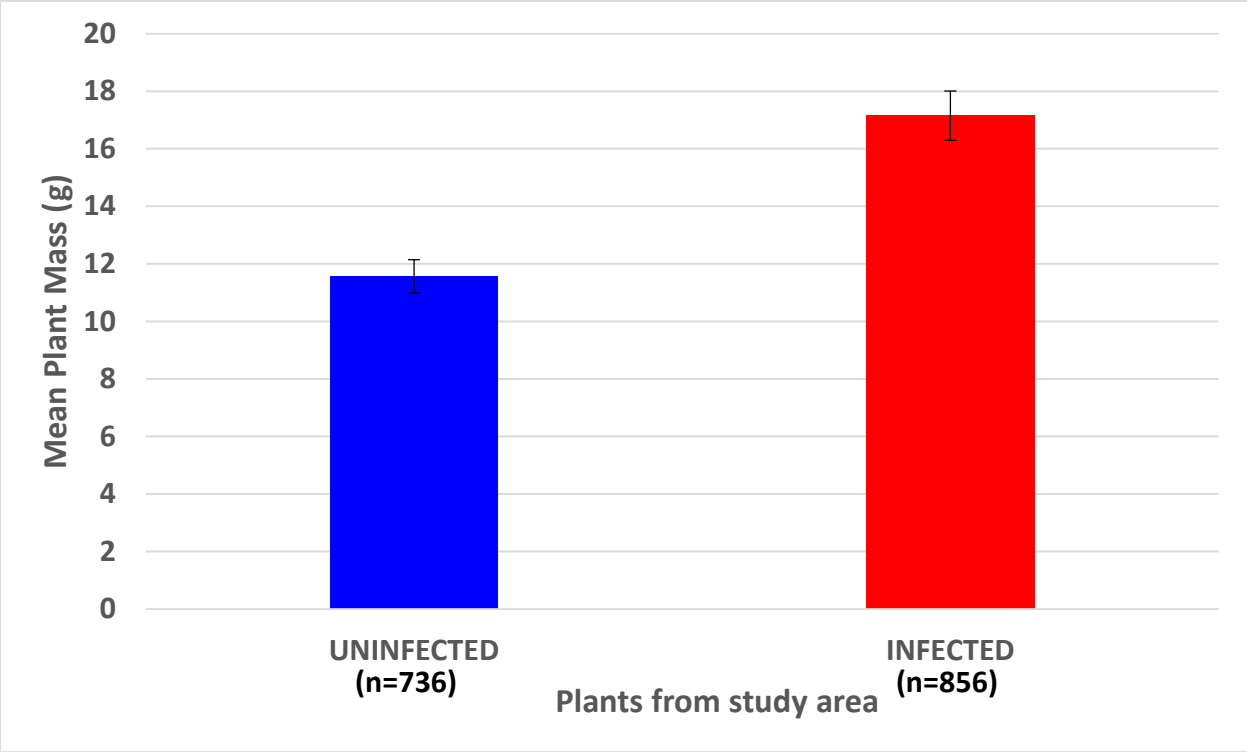

Figure 5: Average plant mass of infected and uninfected plants from the study site (t=2.2580, d.f =1588, p=0.0241)

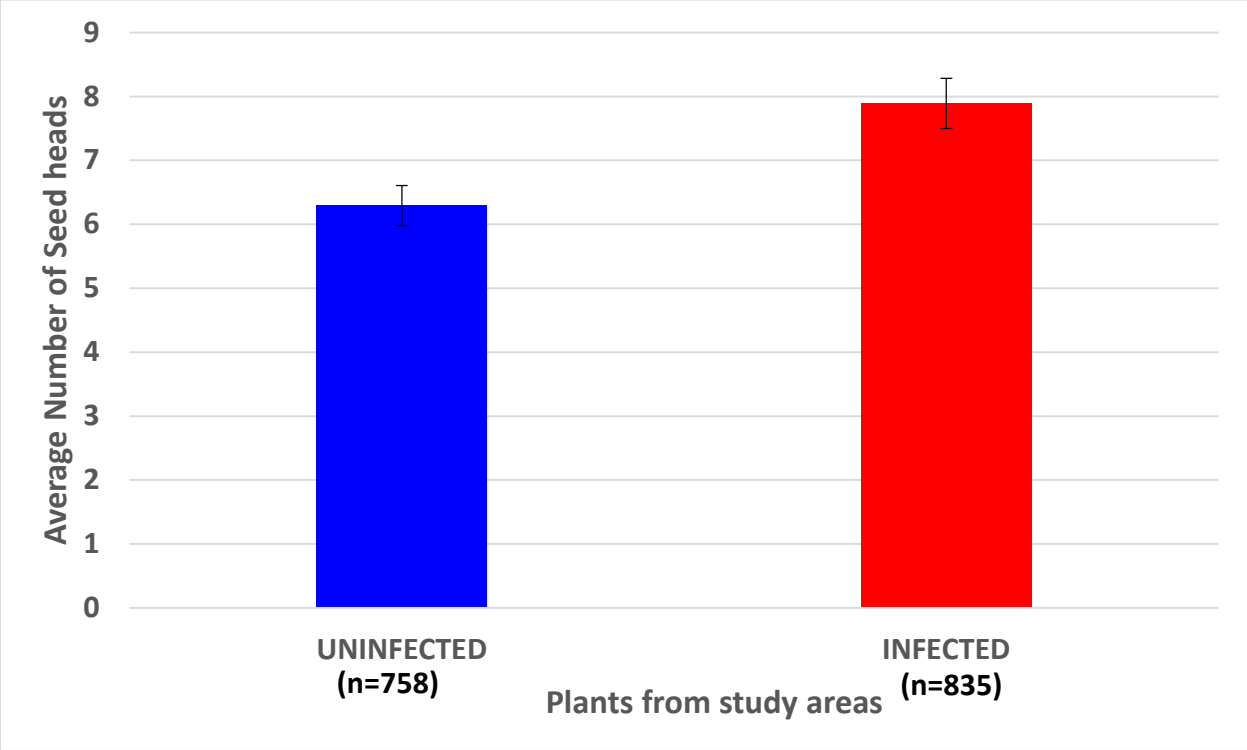

Figure 4: Average number of seed heads of infected and uninfected plants from the study site (t=2.4501, d.f. =1591, p=0.0144)

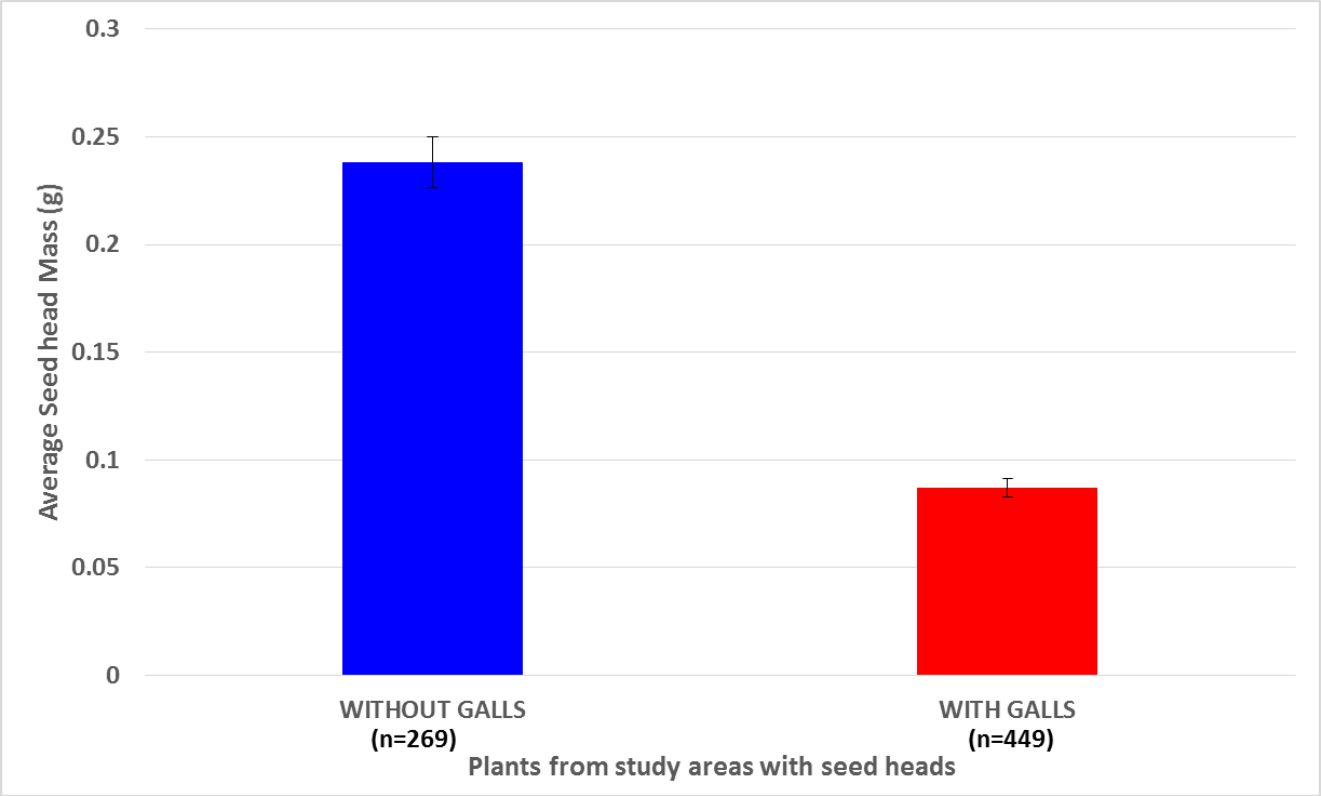

Figure 6: Average seed head mass of plants with and without galls from the study site (t=4.2510, d.f.=716, p<0.001)

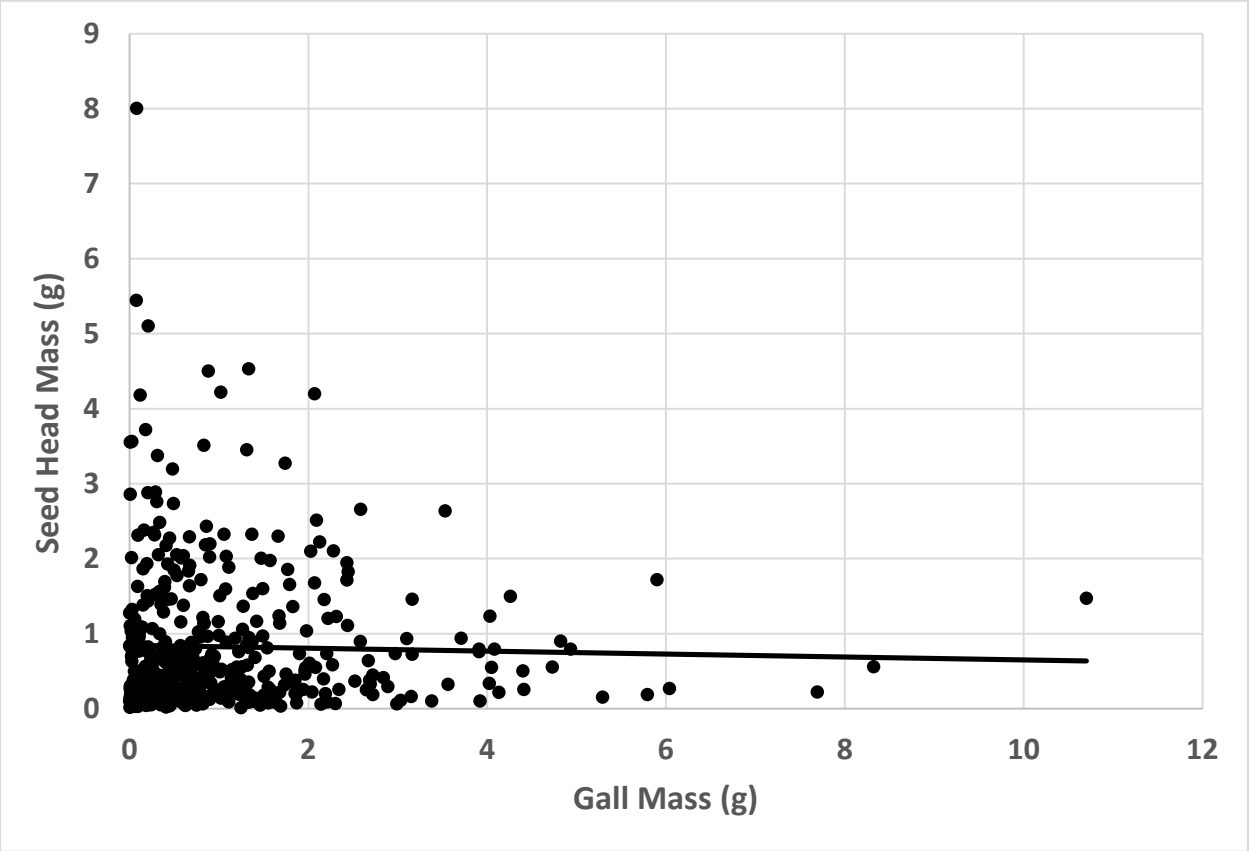

Figure 7: Relationship between seed head weight and gall mass of infected plants from experimental areas (R^2 = 0.00064, d.f. = 1445, p = 0.5942)