

CANADA THISTLE DECLINE, A BIOCONTROL OPPORTUNITY?

by

Dipiza Oli

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ABSTRACT

Canada thistle (*Cirsium arvense*) poses a significant challenge in organic production systems, often forming expansive colonies that reduce crop yield and prevent effective harvest. To control this pest, our program seeks to weaponize thistle pathogens associated with colony collapse for use as bio-herbicides. Pathogens associated with colony collapse were *Alternaria* spp, *Fusarium oxysporum*, and *Fusarium redolens*. Controlled experiments, both in greenhouse and field conditions, were conducted to assess the pathogenicity and efficacy of the *Fusaria* isolates, while ensuring crop safety for Montana's predominant crops. Both *F. oxysporum* and *F. redolens* exhibited strong virulence, reducing thistle vigor by over 70% compared to untreated controls. Koch's postulates were fulfilled, confirming their pathogenicity. For the efficacy of the isolates greenhouse trials demonstrated a significant reduction in thistle health following inoculation with *Fusarium* species ($p < 0.001$). While *Fusarium oxysporum* isolates had no adverse effects on Montana's crops, *Fusarium redolens* isolate significantly reduced the emergence, vigor and dry weight of wheat, lentil, and chickpea in crop safety trials. Field trials showed no crop damage from the inoculation of these *Fusarium* species. We tested the field efficacy of three *F. oxysporum* isolates Bio-02, Bio-05, Bio-15, applied singly and in two-way combinations, at two locations: Highwood and Big Timber, Montana in the year 2023 and an additional Bio-42 isolate in the year 2024 in Highwood and Great Falls. In the year 2023, at Highwood, isolates Bio-02 and Bio-05 reduced thistle stands by 79.2% and 61.2%, respectively ($p = 0.023$). A negative correlation were observed between the reemergence of thistle in Highwood and necrosis in Big Timber ($R^2 = 0.18$, $p = 0.01$) in the year 2023. This research highlights the potential of *Fusarium* species to provide organic farmers with an effective, sustainable alternative to synthetic herbicides for controlling Canada thistle, advancing the goal of environmentally responsible agriculture in Montana.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Introduction

Canada thistle (*Cirsium arvense*) is a highly invasive perennial weed known for its aggressive growth and resilience. It belongs to the Asteraceae family within the tribe Cardueae. The weed is native to the eastern Mediterranean. It has become a significant pest in North America, where it was unintentionally introduced in the 17th century probably through contaminated crop seeds (Hodgson, 1968). It was first reported in the northeastern United States and southeastern Canada, spreading rapidly across the continent. By 2003, Canada thistle had infested in more than 5 million hectares of pasture and wildland. It is currently considered “noxious” in 33 states (USDA-NRCS, 2016), with major infestations in the northern Great Plains, the Midwest, and the Pacific Northwest (Dewey, 1901; Carter & Lym, 2017). Its ability to thrive in various conditions, from agricultural fields to natural ecosystems, has facilitated its widespread distribution. Today, it is considered one of the most problematic invasive weeds in North America, posing significant challenges to both natural and managed ecosystems (Cripps et al., 2011).

Taxonomy and Biology

Canada thistle differs from other members of the *Cirsium* genus, commonly referred to as thistles, by its extensive root system and modes of reproduction. Although most of these thistles are well known for their seed dispersal, Canada thistle reproduces both through seed reproduction and vigorous root-based vegetative reproduction, making it highly invasive. While

Canada thistle is in the same genus, *Cirsium*, with others such as musk thistle (*Cirsium vulgare*) and bull thistle (*Cirsium lanceolatum*), it differs from other species in the genus in that it forms very dense, interconnected colonies by means of horizontal root spread, and its leaves are much less spiny than those of other thistle types. The species name given to Canada thistle is *Cirsium arvense*, where ‘*arvense*’ refers to its habitual presence in cultivated fields and disturbed areas (Moore & Frankton, 1965). Other features that distinguish Canada thistle from other *Cirsium* and *Carduus* species include (i) small dioecious flower heads less than 2.5 cm in diameter, and (ii) a conspicuous lack of spiny-winged stems. There are four accepted types of Canada thistle differentiated by differences in leaf morphology: *var. vestitum* (leaves gray tomentose below), *var. horridum* (leaves glabrous below, thick, wavy, with many marginal spines), *var. integrifolium* (leaves glabrous below, thin, flat and either entire or shallowly pinnatifid) and *var. arvense* (leaves glabrous below, thin, flat, ranging from shallowly to deeply pinnatifid (Moore, 1975). The morphology of *C. arvense* can change based on environmental conditions, and various ecotypes may respond differently to management practices (Hodgson, 1970).

Canada thistle exhibits a complex life cycle that makes it very adaptable to different environments, thereby assuring its survival and propagation. Most plants are from one to one and a half meters in height, with the stems slightly hairy, ridged, and branching, while the leaves are lance-shaped and spiky, reaching up to 20 centimeters long (Royer & Dickinson, 1999). In mid-summer, stems end in clusters of pink or purple flowers. They are insect-pollinated and may produce between 1500 to 5,000 seeds (Kurtz, 2013). Seeds are small, light and have a pappus (a tuft of hairs) allowing them to be dispersed by the wind. Canada thistle seeds can remain viable in soil for up to 20 years, contributing to its persistence (Kurtz, 2013). Following germination,

usually late fall or early spring, a seedling develops a deep taproot for storage of the key nutrient. The first year, the plant overwinters as a rosette and the following spring, the growth resumes when the plant forms new shoots rising upward and horizontal lateral roots that spread outward across the field. An individual plant's root system can spread 5 meters horizontally and 2 meters vertically in the soil in a given year, forming an immense network of roots. Even the smallest root pieces in this network can regrow into whole plants; hence Canada thistle has high tolerance to mechanical control (Nadeau and Vanden, 2017).

Canada Thistle Impact on Crops

Canada thistle competes intensely with crops for light, water, and nutrients leading to significant yield losses ranging from 15-70% (Moyer et al., 1991; McLennan et al., 1991; O'Sullivan et al., 1985; Tiley, 2010). Even at a low density of just 10 Canada thistle shoots per square meter, spring wheat (*Triticum aestivum* L.) experiences a 10% reduction in yield (Donald and Khan, 1992). As the density of Canada thistle continues to increase over time, crop yields decrease further (Jacobs et al., 2006). The extensive root system allows Canada thistle to access deep soil layers, drawing water and nutrients that are often unavailable to crops. Its ability to store energy in extensive root and rhizomatous structures allows Canada thistle to recover quickly from management disturbances (Ringselle et al., 2021). This competitive advantage is especially pronounced in low-water conditions, leading to even greater reductions in crop yield and quality (Beck, 2018).

Canada thistle also poses challenges through its allelopathic properties (Stachon et al., 1980). Extracts from the roots and foliage of thistle have been found to inhibit the germination and growth of pasture and several crops including wheat, beans, sugar beets and alfalfa. (Wilson,

1981; Pilipavičius & Romaneckas, 2014), as well as its own seedlings (All, 1975). Canada thistle infestations are often associated with low species diversity. Studies have demonstrated that Canada thistle residues inhibit the growth of redroot pigweed (*Amaranthus retroflexus*) and green foxtail (*Setaria viridis*) in agricultural fields (Stachon & Zimdahl, 1980). In heavily infested fields, wheat yields may decrease by up to 40%, particularly during dry seasons, as the weed out-competes crops for moisture and nutrients. (Jacobs et al., 2006). Pulse crops, such as lentil and pea, are also significantly affected. In a 2018 survey, 64% of Montana ranchers rated Canada thistle as the most troublesome weed, stating it is one of the main reasons behind poor livestock performance (Mangold et al., 2018). Its allelopathic properties may also alter soil microbial communities, disrupting nutrient cycling and further limiting the growth of other plant species (Verbeek & Kotanen, 2019). These combined effects make Canada thistle a particularly difficult weed to manage in crop systems. The invasion of Canada thistle not only reduces plant biodiversity but also disrupts habitats for beneficial insects and wildlife. As a result, ecosystem services—such as pollination and natural pest control—are diminished (Donald, 1994), exacerbating the negative impacts of this invasive species on agricultural systems.

Chemical Control of Canada Thistle

The choice of herbicide and precise application timing, particularly during the early growth stage or before the flower bud stage, are critical for successful control (Boerboom & Wyse, 1988; Darwent et al., 1994). Once the root network extends, the plant's stored carbohydrate reserves can fuel new shoot growth, even after herbicide application (Haderlie, 2021). Selective herbicides like clopyralid are more effective when applied to rosettes than to bolted plants (Miller and Lym, 2017). Commonly used herbicides for Canada thistle include

glyphosate, 2,4-D, dicamba, clopyralid, and aminopyralid (Miller and Lym, 2017; Enloe et al., 2007). Aminopyralid when applied at lower rates has been found to be more effective than the standard herbicide in the Great Plains (Enloe et al., 2007). The increasing prevalence of herbicide-resistant Canada thistle populations presents a significant challenge, underscoring the need for integrated weed management strategies that combine chemical, mechanical, and cultural control methods (Heap, 2014). Canada thistle has exhibited reduced susceptibility to MCPA (phenoxy-acetic acid) since the 1950s, particularly in high-input farmlands, where herbicides are used intensively (Fogelfors & Lundkvist, 2008). Similar effects were observed by the ALS-inhibitors like triasulphuron, chlorsulphuron and tribenuron-methyl (Frank & Tworowski, 1994; Solymosi & Nagy, 1998), photosystem II inhibitors triazines (Bachthaler et al., 1983) and clopyralid (Frank & Tworowski, 1994) on the dry matter production of *C. arvensis* above ground. This highlights the urgent need for diversified management approaches to mitigate the spread of herbicide-resistant populations and ensure effective long-term control of Canada thistle.

Cultural and Mechanical Control of Canada Thistle

Maintaining optimal soil fertility and moisture is essential for the successful establishment and growth of crops or pasture plants in areas where Canada thistle has become dominant. Without these practices, the competitive nature of Canada thistle can hinder crop growth by monopolizing resources like water and nutrients. Ensuring proper soil management helps reduce the advantage Canada thistle has, creating an environment where winter annual grains and perennial forage plants can thrive and effectively compete against this invasive weed (Jacobs et al., 2006). In organic farming systems, crop cultivation and mechanical tillage are among the most commonly used strategies for weed control (Liebman and Davis, 2009; Orloff et

al., 2018). By lowering weed vigor and stopping the development of weed seeds, cultivation and tillage can provide crops with a competitive advantage (Bowman and Halvorson, 1997). With at least one cultivation every 28 days throughout a growing season, frequent seasonal plowing and cultivations have the ability to effectively manage *C. arvensis* (Stevens 1846; Tiley, 2010; Alley, 1981). Reducing *C. arvensis* may depend on when the plant is cultivated (Arny, 1932; McAllister and Haderlie, 1985). Early June, when *C. arvensis* begins to flower, the assimilated reserves in the root system are at their lowest (Hodgson 1968, McAllister and Haderlie 1985). The thistle plant becomes weaker as its reproductive parts develop because of the substantial consumption of root assimilate reserves (Heinisch, 1931). Thistle plants are therefore thought to be equally vulnerable to various forms of disturbance in May and June, such as deep plowing. However, prolonged and heavy tillage practices may lead to soil aggregate disintegration, water and wind erosion, a decrease in organic matter, and water loss from evaporation and infiltration.

Furthermore, Orloff demonstrated that mechanical weed management was not effective when applied alone. Rather, it seems that the best results from mechanical management came from its combination with two or more other management strategies such as biocontrol, crop competition, solarization, grazing, flaming, crop diversification, mowing or shading (Orloff et al., 2018).

Another most common mechanical methods used by growers for Canada thistle control is hoeing and mowing (Hurrell and Bourdot, 1996, Bacher et al. 1997). These methods do not effectively eliminate thistle as they fail to eradicate the root system. Instead, they may actually facilitate the clonal expansion of thistle from the small root fragments left behind after cutting. Mowing might even benefit thistle by reducing competition from other plant species (Edwards et al., 2000).

Biological Control of Canada thistle

Biological control of weeds is the use of live organisms that reduce weed densities and thus crop damage. The two most popular approaches to implement biocontrol are the (1) classical and (2) inundative strategies. The classical biocontrol is "the purposeful release of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control" (Eilenberg et al., 2001). The applied weed biocontrol agent and the weed itself have an exotic origin in classical traditional biological control. This approach is only applied if the weeds are introduced by accident. It is becoming more and more crucial to employ biological control agents as part of an integrated, sustainable approach to managing plant weeds. Unlike chemical herbicides, these biological agents target weed in a more sustainable manner, reducing reliance on synthetic chemicals and lowering the risk of environmental damage.

Different biological control agents have been identified and used to manage Canada thistle, including insects, fungi, and other pathogens. Several insects have been introduced as biocontrol agents for Canada thistle includes stem-mining weevil, gall fly, rosette weevil and flea beetles. Biocontrol efforts against Canada thistle began in the late 19th and early 20th centuries in Europe, where the weed is native. Some of these, in the native range, have been noted by researchers and land managers as potentially important natural enemies and elements in the control of the plant, like the Canada thistle stem-mining weevil. The thistle stem-mining weevil, *Hadroplontus litura* lays eggs by boring holes into the stems of thistle; the larvae help weaken the thistle— even at very early developmental stages—and reduce its growth and eventual number of flowers and seed heads (Zwolfer & Harris, 1966; Peschken & Wilkinson, 1981). The importance of this stem-mining weevil in biocontrol of the Canada thistle was documented since the late 1960s, with confirmation by other researchers in late 90s and early 2000s (Halsey, 1992;

Larson et al., 2005). Later, in the mid-20th century, researchers from North America began to get interested in utilizing some of these natural enemies from Europe to reduce infestations of the Canada thistle. They introduced Canada thistle gall fly (*Urophora cardui*), and the Canada thistle rosette weevil (*Trichosirocalus horridus*). These insects target the plant at different parts, thus combining effect contributing towards its control. As of now, there are three officially permitted biological control agents for managing Canada thistle which are stem gall fly (*Urophora cardui*), stem-mining weevil (*Hadroplontus litura*) and the thistle flea beetles (*Altica carduorum*) (Pitcarn, 2021). However, Cripps et al. (2011) concluded that Canada thistles were not significantly influenced by any of the herbivorous arthropod species in their review. The rust fungus *Puccinia punctiformis* that infects Canada thistle leaves became the first plant pathogen proposed as weed control agent in 19th century (Halsted, 1894). *Puccinia punctiformis* is responsible for causing two types of infections, namely local and systemic infections. Local infections result in small lesions on thistle leaves and have a minimal impact on the plant's performance (Kluth et al., 2005). In contrast, systemic infections typically lead to the death of infected shoots within a few months, often before flowering (French and Lightfield, 1990). Previous research has found that artificial inoculation generally results in systemic infection rates of 20 to 50% (Van den et al., 1987; French et al., 1988; Frantzen, 1994; Wandeler and Bacher 2006; Müller et al., 2011), which is considered insufficient for effectively suppressing *C. arvense* (Van Leest and Scheepens, 1994).

Fusarium as a Bioherbicide

Fusarium is one of the most extensively researched genera of filamentous fungi, for its potential use as a bioherbicide. Due to their host specificity, certain *Fusarium* species can target

specific weed species without harming non-target plants or crops, making them an attractive option for integrated weed management systems (Vam Dam et al., 2018). This host-specificity reduces the risk of off-target effects, which is a significant concern with traditional chemical herbicides. Several studies have explored the application of *Fusarium* species in both terrestrial and aquatic environments. For instance, *Fusarium spp.* have been used to control alligator weed (*Alternanthera philoxeroides*) in various agricultural environments (Tan et al., 2002). *Fusarium solani* has demonstrated up to 99% effectiveness in controlling Texas gourd in field trials when applied as a granular inoculum (Boyette et al., 1984). Other species such as *Fusarium avenaceum* were found to inhibit the growth of specific weeds like spotted knapweed and wild oat through direct pathogenic effects (Pleban & Strobel, 1998). These studies highlight the potential of *Fusarium* to act as an effective bioherbicide under the right conditions, though further research is needed to optimize delivery systems for broader applications.

Potential of *Fusarium oxysporum* for Weed Control

Fusarium oxysporum has shown considerable promise as a biocontrol agent against several noxious weed species. Its long persistence in the soil, or long half-life, allows it to provide extended control over weed populations, preventing regrowth after initial treatment. Early studies, such as those conducted in Hawaii in the 1940s, demonstrated the effectiveness of *F. oxysporum* as a mycoherbicide to control the invasive tree cactus (*Opuntia megacantha*) (Fullaway, 1954; Wilson, 1969). Several formae speciales of *F. oxysporum* have been successfully deployed in biological control programs, such as *F. oxysporum f.sp. cannabidis* for controlling hemp (Tiourebaev, 2001; Punja, 2018), and *F. oxysporum f.sp. strigae* against striga (Marley et al., 2005; Venne et al., 2009; Lüth et al., 2016). More recently *Fusarium oxysporum*

has been used against parasitic weeds like *Phelipanche ramosa*. Kohlschmid et al. (2020) found reducing emergence of this parasitic weed by 70%. Similarly, *F. oxysporum* strains tested in Italy significantly reduced broomrape (*Orobancha spp.*) infestations by as much as 60% (Boari & Vurro, 2021). These findings underscore the potential of *F. oxysporum* as a powerful tool for sustainable weed management.

Mechanisms and Challenges of *Fusarium oxysporum*

The infection process of *Fusarium oxysporum* begins when its spores adhere to the roots of weeds, where they germinate and form hyphae that penetrate root tissues. The fungus colonizes the plant's vascular system, primarily the xylem, disrupting the flow of water and nutrients, which leads to wilting, chlorosis, and eventually plant death (Fig 1.2) (Ma, 2013).

Fusarium oxysporum also produces toxins that interfere with cellular functions and induce defense responses that further damage the plant (Nelson et al., 1993). The pathogen's virulence is supported by an arsenal of enzymes such as cellulases and pectinases, which degrade plant cell walls and allow the fungus to invade deeper into plant tissues (Michielse & Rep, 2009). While the biological mechanisms of infection are well-understood, several challenges remain in applying *F. oxysporum* as a consistent and reliable bioherbicide. Environmental factors such as soil moisture, temperature, and pH can significantly influence the efficacy of the fungus in field conditions. Moreover, strain specificity can limit its usefulness to only certain weed species, requiring careful selection and testing of strains for each target weed. Despite these challenges, *F. oxysporum* continues to be a promising candidate for weed control in both conventional and organic systems, especially when integrated with other management practices like crop rotation and mechanical tillage.

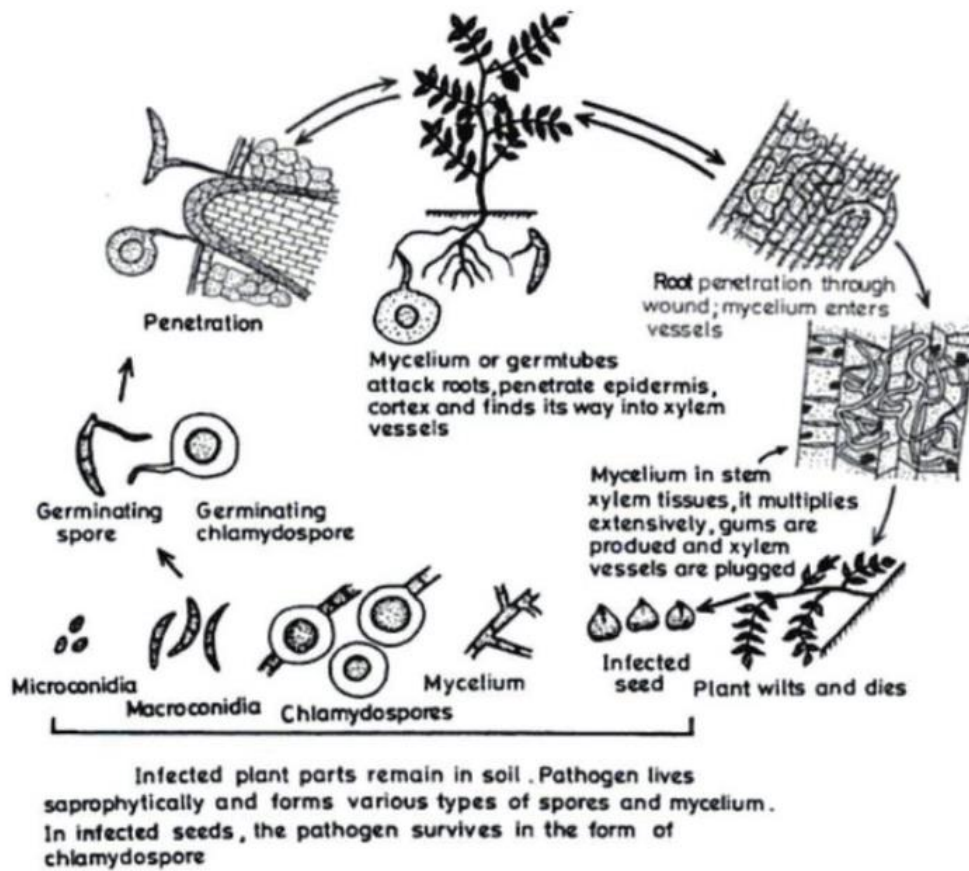


Figure 1.2 Disease cycle of *Fusarium oxysporum* (source: Jalali and Chand, 1992)

References Cited

- All, G.M.B. (1975). The allelopathic activity of Californian thistle (*Cirsium arvense* (L.) Scop.) in Tasmania. *Weed Research*, 15, 77-81. <https://doi.org/10.1111/j.1365-3180.1975.tb01102.x>
- Alley, H. P. (1981). Mechanical, cultural, and chemical control of Canada thistle in small grains and pastures. In *Proc. North Cent. Weed Control Conf*, 36, 176-179.
- Arny, A.C. (1932). Variations in the organic reserves in underground parts of five perennial weeds from late April to November. *Minnesota Agricultural Experiment station's Bulletin* No. 84, 28.
- Bacher, S., Heitzmann, A., Nentwig, W. (1997). Problemunkräuter in ökologischen Ausgleichsflächen im Ackerbau. *Agrarforschung* 4, 65–67.
- Bachthaler, G., Kees, H., & Dinzenhofer, B. (1983). The build-up of resistant lines of arable weeds after continued use of herbicides, especially triazines. Present state of knowledge of causes and practical consequences.
- Beck, K.G., (2018). Canada Thistle -Extension, 3-108
- Boari, Angela., & Maurizio, Vurro. (2004). Evaluation of *Fusarium spp.* And Other Fungi as Biological Control Agents of Broomrape (*Orobanche Ramosa*). *Biological Control*, 30, 212–219, <https://doi.org/10.1016/j.biocontrol.2003.12.003>
- Boerboom, C. M., & Wyse, D. L. (1988). Selective application of herbicides for Canada thistle (*Cirsium arvense*) control in birdsfoot trefoil (*Lotus corniculatus*). *Weed Technology*, 2(2), 183-186.
- Bowman, R. A., & Halvorson, A. D. (1997). Crop rotation and tillage effects on phosphorus distribution in the central Great Plains. *Soil Science Society of America Journal*, 61(5), 1418-1422.
- Boyette, C. D., Templeton, G. E., & Oliver, L. R. (1984). Texas Gourd (*Cucurbita texana*) Control with *Fusarium solani* f. sp. *cucurbitae*. *Weed Science*, 32(5), 649–655. doi:10.1017/S0043174500059737.
- Carter, TR., and Lym, R.G. (2017). Canada thistle (*Cirsium arvense*) affects herbage production in the Northern Great Plains. *Invasive Plant Science and Management*. 10(4): 332-339. doi:10.1017/inp.2017.34.
- Cripps, M. G., Gassmann, A., Fowler, S. V., Bourdôt, G. W., McClay, A. S., & Edwards, G. R. (2011). Classical biological control of *Cirsium arvense*: lessons from the past. *Biological Control*, 57(3), 165-174.

- Darwent, A. L., Kirkland, K. J., Baig, M. N., & Lefkovitch, L. P. (1994). Preharvest applications of glyphosate for Canada thistle (*Cirsium arvense*) control. *Weed technology*, 8(3), 477-482.
- Dewey, L.H. (1901). Canada thistle [*Carduus arvensis* (L.) Robs.]. Washington, DC: USDA Division of Botany, 27. 14.
- Donald, W.W. (1994). Geostatistics for mapping weeds, with a Canada thistle (*Cirsium arvense*) patch as a case study. *Weed Science*, 42: 648-657.
- Donald, W. W., & Khan, M. (1992). Yield loss assessment for spring wheat (*Triticum aestivum*) infested with Canada thistle (*Cirsium arvense*). *Weed Science*, 40(4), 590-598.
- Edwards, GR., Bourdôt. G.W., and Crawley. MJ. (2000). Influence of herbivory, competition and soil fertility on the abundance of *Cirsium arvense* in acid grasslands. *Journal of Applied Ecology*, 37: 321–334. doi: 10.1046/j.1365-2664.2000.00495.x
- Eilenberg, J., Hajek. A., and Lomer. C. (2001). Suggestions for unifying the terminology in biological control. *Biocontrol Sci Technol*, 46:387–400.
- Enloe, S. F., Lym, R. G., Wilson, R., Westra, P., Nissen, S., Beck, G., and Halstvedt, M. (2007). Canada thistle (*Cirsium arvense*) control with aminopyralid in range, pasture and noncrop areas. *Weed Technology*, 21(4), 890–894. doi:10.1614/WT-07-004.1
- Fogelfors, H., & Lundkvist, A. (2008). Selection in *Cirsium arvense* (L.) Scop. and *Sonchus arvensis* L.: Susceptibility to MCPA on different types of farmland in Sweden. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, 58(1), 82-87
- Frank, J. R., & Tworkoski, T. J. (1994). Response of Canada thistle (*Cirsium arvense*) and leafy spurge (*Euphorbia esula*) clones to chlorsulfuron, clopyralid, and glyphosate. *Weed technology*, 8(3), 565-571.
- Frantzen, J. (1994). The role of clonal growth in the pathosystem *Cirsium arvense* *Puccinia punctiformis*. *Canadian Journal of Botany*, 72: 832–836. doi: 10.1139/b94-107
- French, R.C., and Lightfield. AR. (1990). Induction of systemic aecial infection in Canada thistle (*Cirsium arvense*) by teliospores of *Puccinia punctiformis*. *Phytopathology*, 80: 872–877. doi: 10.1094/Phyto-80-872
- French, R.C., Turner, S.K., Sonnett, P.E., Pfeffer, P., and Piotrowski, E. (1988). Properties of an extract from Canada thistle roots that stimulates germination of dormant teliospores of Canada thistle rust (*Puccinia punctiformis*). *Journal of Agriculture and Food Chemistry* 36: 1043–1047. doi: 10.1021/jf00083a034
- Fullaway, D.T. (1954). Biological control of cactus in Hawaii. *Journal of Economic Entomology*, 47:696-700

- Haderlie, L. C., McAllister, R. S., Hoefler, R. H., & Leino, P. W. (1992). Canada thistle control. In *Noxious Range Weeds*, 260-263
- Halsey, W. S. (1992). Responses of Canada thistle to defoliation and to damage from a stem-mining weevil in Utah.
- Halsted, B. D. (1894). Weeds and their most common fungi. *New Jersey Exp. Stn. Rep.*, 1002
- Heap, I. (2014). Global perspective of herbicide-resistant weeds. *Pest Manag. Sci.* 70, 1306–1315. doi:10.1002/ps.3696
- Heinisch, O. (1931). Die Ackerkratzdistel. *Cirsium arvense* (L.) Scop. In Roemer, Th. (ed.) *Archiv für Pflanzenbau*. Verlag von Julius Springer, Berlin, 348–420.
- Hodgson, J.M. (1968). The nature, ecology, and control of Canada thistle. Technical Bulletin of the United States Department of Agriculture, No. 1386.
- Hodgson, J. M. (1970). The response of Canada thistle ecotypes to 2, 4-D, amitrole, and intensive cultivation. *Weed Science*, 18(2), 253-255.
- Hurrell, G.A., and Bourdot, G.W. (1996). *Sclerotinia sclerotiorum* and mowing independently reduce Californian thistle in a sheep pasture. *New Zealand Plant Protection Society*, Rotorua, NZ. ISSG (2011) Invasive species specialist group.
- Jacobs, J., Sciegienka, J., & Menalled, F. D. (2006). Ecology and Management of Canada Thistle (*Cirsium arvense* (L.) Scop.). US Department of Agriculture, Natural Resources Conservation Service.
- Jalali, B.L., and Chand, H. (1992). Chickpea wilt. In: *Plant Diseases of International Importance*. Vol. 1. Diseases of Cereals and Pulses. U. S. Singh, A.N., Mukhopadhyay, J. Kumar., and H. S. Chaube, eds. Prentice Hall, Englewood Cliffs, NY, USA. p. 429-444
- Kluth, S., Kruess, A., and Tschardt, T. (2005). Effects of two pathogens on the performance of *Cirsium arvense* in a successional fallow. *Weed Research* 45: 261–269. doi:10.1111/j.1365- 3180.2005.00463.x
- Kohlschmid, E., Sauerborn, J., & Müller-Stöver, D. (2009). Impact of *Fusarium oxysporum* on the holoparasitic weed *Phelipanche ramosa*: biocontrol efficacy under field-grown conditions. *Weed research*, 49, 56-65.
- Kurtz, Cassandra M. (2013). An assessment of invasive plant species monitored by the Northern Research Station Forest Inventory and Analysis Program, 2005 through 2010, USDA, 43136, <https://doi.org/10.2737/nrs-gtr-109>
- Larson, G. E., Wittig, T. A., Higgins, K. F., Turnipseed, B., & Gardner, D. M. (2005). Influence of biocontrol insects on Canada thistle: seed production, germinability, and viability.

- Liebman, M., & Davis, A. S. (2009). Managing weeds in organic farming systems: an ecological approach. *Organic farming: the ecological system*, 54, 173-195.
- Lüth, P., Nzioki, H. S., Sands Baker, C., & Sands, D. C. (2024). A microbial bioherbicide for *Striga hermonthica* control: production, development, and effectiveness of a seed coating agent. *Pest Management Science*, 80(1), 149-155.
- Ma, L.-J., Geiser, D. M., Proctor, R. H., Rooney, A. P., O'Donnell, K., Trail, F., & Kistler, H. C. (2013). *Fusarium* pathogenomics. *Annual Review of Microbiology*, 67, 399-416.
- Mangold, J. M., Fuller, K. B., Davis, S. C., & Rinella, M. J. (2018). The Economic Cost of Noxious Weeds on Montana Grazing Lands. *Invasive Plant Science and Management*, 11(2), 96–100. doi:10.1017/inp.2018.10
- Marley, P. S., Kroschel, J., & Elzien, A. (2005). Host specificity of *Fusarium oxysporum* Schlect (isolate PSM 197), a potential mycoherbicide for controlling *Striga* spp. in West Africa. *Weed research*, 45(6), 407-412.
- McAllister, R.S., and Haderlie, L.C. (1985). Seasonal variations in Canada thistle (*Cirsium arvense*) root bud growth and root carbohydrate reserves. *Weed Science* 33:44–49.
- McLennan, B. R., Ashford, R., & Devine, M. D. (1991). *Cirsium arvense* (L.) Scop. competition with winter wheat (*Triticum aestivum* L.). *Weed Research*, 31(6), 409-415.
- Michielse, C. B., & Rep, M. (2009). Pathogen profile update: *Fusarium oxysporum*. *Molecular Plant Pathology*, 10(3), 311-324.
- Miller, B. R., & Lym, R. G. (2017). Using the rosette technique for Canada thistle (*Cirsium arvense*) control in row crops. *Weed Technology*, 12(4), 699–706. doi:10.1017/S0890037X00044572
- Moore, R. J. (1975). The biology of Canadian weeds: *Cirsium arvense* (L.) Scop. *Can. J. Plant Sci.* 55, 13, 1033–1048. doi:10.4141/cjps75-163
- Moore, R.J., & Frankton, C. (1965). Cytotaxonomy of *Cirsium hookerianum* and related species. *Canadian Journal of Botany*, 43, 597–613.
- Moyer, J. R., Schaalje, G. B., & Bergen, P. (1991). Alfalfa (*Medicago sativa*) seed yield loss due to Canada thistle (*Cirsium arvense*). *Weed Technology*, 5(4), 723-728.
- Müller, E., Jud, P., and Nentwig, W. (2011). Artificial infection of *Cirsium arvense* with the rust pathogen *Puccinia punctiformis* by imitation of natural spore-transfer of the weevil *Ceratopion onopordi*. *Weed Research*, doi: 10.1111/j.1365-3180.2010.00839.x
- Nadeau, L. B., and H, Vanden. (1989). The root system of Canada thistle. *Canadian Journal of Plant Science*, 69(4), 1199–1206, <https://doi.org/10.4141/cjps89-142>

- Nelson, P. E., Desjardins, A. E., & Plattner, R. D. (1993). Fumonisin, mycotoxins produced by *Fusarium* species: biology, chemistry, and significance.
- Orloff, N., Mangold, J., Miller, Z., & Menalled, F. (2018). A meta-analysis of field bindweed (*Convolvulus arvensis* L.) and Canada thistle (*Cirsium arvense* L.) management in organic agricultural systems. *Agriculture, Ecosystems & Environment*, 254, 264-272.
- O'Sullivan, P.A., Kossatz, V.C., Weiss, G.M., and Dew, D.A. (1982). An approach to estimating yield loss of barley due to Canada thistle. *Can J Plant Sci* 62:125-131.
<https://doi.org/10.4141/cjps82-105>
- O'Sullivan, P.A., Weiss, G.M., and Kossatz, V.C. (1985). Indices of competition for estimating rapeseed yield loss due to Canada thistle. *Can J Plant Sci* 65:145-149.
<https://doi.org/10.4141/cjps85-020>
- Peschken, D. P., & Wilkinson, A. T. S. (1981). Biocontrol of *C. arvense* (*Cirsium arvense*): releases and effectiveness of *Ceutorhynchus litura* (Coleoptera: Curculionidae) in Canada. *Canadian Entomologist*, 113(9), 777-785.
- Pilipavičius, V., and Romanekas, K. (2014). Allelopathic activity of creeping thistle water extracts on germination and early growth of winter wheat. *Bulgarian Journal of Agricultural Science* 20(3):607-612 doi: 20.500.12259/87144
- Pleban, S., & Strobel, G. A. (1998). Rapid evaluation of *Fusarium* spp. as a potential biocontrol agent for weeds. *weed Science*, 46(6), 703-706.
- Punja, Z. K., & Rodriguez, G. (2018). *Fusarium* and *Pythium* species infecting roots of hydroponically grown marijuana (*Cannabis sativa* L.) plants. *Canadian Journal of Plant Pathology*, 40(4), 498-513.
- Ringselle, B., Brandsæter, L. O., Mangerud, K., & Bergkvist, G. (2023). Vertical rhizome disking to reduce *Elymus repens* (quackgrass) abundance in grass-clover leys. *Crop Protection*, 172, 106301.
- Royer, F., and R. Dickinson. (1999). Weeds of the Northern U.S. and Canada. The University of Alberta press. 434
- Solymosi, P., & Nagy, P. (1998). ALS-resistance in *Cirsium arvense* (L.) scop.
- Stachon, W. J., & Zimdahl, R. L. (1980). Allelopathic Activity of Canada Thistle (*Cirsium arvense*) in Colorado. *Weed Science*, 28(1), 83-86. doi:10.1017/S004317450002782X
- Stevens, A. (1846). Extirpation of Canada thistles. *Extirpation of Canada thistles*. 406-428.
- Tan, W. Z., Li, Q. J., & Qing, L. (2002). Biological control of alligatorweed (*Alternanthera philoxeroides*) with a *Fusarium* sp. *Bio Control*, 47, 463-479.

- Tiley, G.E. (2010). Biological flora of the British Isles: *Cirsium arvense* (L.) Scop. J Ecol 98(4):938–983. <https://doi.org/10.1111/j.1365-2745.2010.01678.x>
- Tiourebaev, K. S., Semenchenko, G. V., Dolgovskaya, M., McCarthy, M. K., Anderson, T. W., Carsten, L. D., Pilgeram, A.L., & Sands, D. C. (2001). Biological control of infestations of ditchweed (*Cannabis sativa*) with *Fusarium oxysporum* f. sp. *cannabis* in Kazakhstan. Biocontrol science and technology, 11(4), 535-540.
- Van Dam, P., De Sain, M., Ter Horst, A., Van Der Gragt, M., & Rep, M. (2018). Use of comparative genomics-based markers for discrimination of host specificity in *Fusarium oxysporum*. Applied and environmental microbiology, 84(1), e01868-17.
- Van den, Ende G., Frantzen, J., Timmers, T., (1987) Teleutospores as origin of systemic infection of *Cirsium arvense* by *Puccinia punctiformis*. Netherland Journal of Plant Pathology 93: 233–239. doi: 10.1007/BF01998251
- Van Leest, AS., and Scheepens, P.C. (1994). Effectiveness of the rust fungus *Puccinia punctiformis* as a possible biological control agent against Canada thistle (*Cirsium arvense*). Rapport- DLO Instituut voor Agrobiologisch en Bodemvruchtbaarheidsonderzoek zoek 25: 1–19.
- Venne, J., Beed, F., Avocanh, A., & Watson, A. (2009). Integrating *Fusarium oxysporum* f. sp. *strigae* into cereal cropping systems in Africa. Pest Management Science: Formerly Pesticide Science, 65(5), 572-580.
- Verbeek, J.D., and Kotanen, P.M. (2019). Soil-mediated impacts of an invasive thistle inhibit the recruitment of certain native plants. Oecologia, 190, 619–628. <https://doi.org/10.1007/s00442-019-04435-8>
- Wandeler, H., and Bacher, S. (2006). Insect-transmitted urediniospores of the rust *Puccinia punctiformis* cause systemic infections in established *Cirsium arvense* plants. Phytopathology 96: 813–818. doi: 10.1094/PHYTO-96-0813
- Wilson, C.L. (1969). Use of plant pathogens in weed control. Annual Review of Phytopathology 7:411-434.
- Wilson, R. G. (1981). Effect of Canada thistle (*Cirsium arvense*) residue on growth of some crops. Weed Science, 29(2), 159-164.
- Zwolfer, H., & Harris, P. (1966). *Ceutorhynchus litura* (F.) (Col. Curculionidae), a potential insect for the biological control of thistle, *Cirsium arvense* (L.) Scop. in Canada. Canadian Journal of Zoology, 44(1), 3–38.

CHAPTER TWO

PATHOLOGY OF CANADA THISTLE

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Dipiza Oli

Contributions: Field planting, data and sample collection, data analysis, Writing-original draft

Co-Author: Alan T Dyer

Contributions: Resources, Conceptualization, Field Supervision, Data Validation, Review and editing manuscript

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Dipiza Oli, Alan T Dyer

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Introduction

Canada thistle (*Cirsium arvense*) is a highly resilient perennial weed that poses significant challenges to agriculture and natural ecosystems due to its aggressive growth and adaptability. It is native to the eastern Mediterranean and was unintentionally introduced to North America as a seed contaminant (Hodgson, 1968). Its dual reproductive strategies—prolific seed production and extensive vegetative propagation through a deep-rooted, creeping root system—make it notoriously difficult to control. Each plant can produce thousands of wind-dispersed seeds, which allows it to invade a wide variety of landscapes (Kurtz, 2013). Once established, its root system, which can extend several meters deep, enables it to access water and nutrients unavailable to many other plants (Donald, 1994).

Losses associated with Canada thistle have been documented for a wide array of crops with losses ranging from 15-70% (Moyer et al., 1991; McLennan et al., 1991; Chad and Edward, 2004; O’Sullivan et al., 1982; Tiley, 2010). Thistle-related losses are positively correlated with weed densities (O’Sullivan et al., 1985). The competition for light, water, and nutrients by Canada thistle directly impacts crop performance. Apart from direct competition, Canada thistle exudes allelochemicals shown to inhibit the germination and growth of wheat, beans, sugar beets and alfalfa (Wilson, 1981; Pilipavičius & Romaneckas, 2014). Its allelopathic characteristics can change soil microbial communities, interfering with nutrient cycling, further restricting the growth of other plant species (Verbeek & Kotanen, 2019). These cumulative impacts make Canada thistle particularly challenging to control. The presence of Canada thistle not only decreases plant diversity but also interrupts habitats essential for beneficial insects and wildlife (Jacobs et al., 2006). Consequently, ecosystem services—such as pollination and natural pest

management—are weakened (Donald, 1994), intensifying the adverse effects of this invasive species on farming systems.

Successful management of Canada thistle requires a multifaceted approach. Maintaining optimal soil fertility and moisture is essential for the successful establishment and growth of crops or pasture plants in areas where Canada thistle has become dominant. Winter annual grains and perennial forage plants are the most effective in competing with Canada thistle (Jim et al., 2006). In organic farming systems, crop cultivation and mechanical tillage are among the most commonly used strategies for thistle control (Liebman and Davis, 2009; Orloff et al., 2018). However, prolonged and heavy tillage practices may lead to soil aggregate disintegration, water and wind erosion, a decrease in organic matter, and water loss from evaporation and reduced infiltration which makes approach undesirable from sustainable farming perspective. Furthermore, Orloff demonstrated that mechanical weed management was not effective when applied alone and other effective approaches are needed (Orloff et al., 2018). For conventional farm systems, thistle control may involve crop cultivation and the use of herbicides such as glyphosate, 2,4-D, dicamba, clopyralid, and aminopyralid (Miller and Lym, 2017; Enloe et al., 2007). Unfortunately, resistance to these products is becoming common (Heap, 2014).

In light of these challenges, biocontrol agents that target Canada thistle have gained interest over the years. Application of insects including stem-mining weevil, gall fly, rosette weevil, and flea beetles have shown some promise (Canada Thistle Biocontrol | Department of Agriculture). Unfortunately, these insects are host dependent and when thistle populations decline, insect populations readily become locally extinct. Plant pathogens have also been of interest with the rust fungus *Puccinia punctiformis* being the most widely studied (Halsted, 1894;

French and Lightfield, 1990; Kluth et al., 2005; Wandeler and Bacher 2006; Müller et al., 2011). Being an obligate pathogen, this rust fungus suffers from similar challenges as insect biocontrols.

More recently, the fungal genera *Fusarium* has been identified in the rhizosphere of Canada thistle, pointing to potential microbial interactions that could be leveraged for biological control (Eberly et al., 2024). However, the pathology of Canada thistle, particularly its interaction with fungi and nematodes remains insufficiently understood. Given the potential for mycoherbicides as a biological control agents, the aim of this chapter was to

1. Isolating and characterizing the pathogens associated with Canada thistle could provide valuable insights into developing more effective management approaches. By isolating the pathogens associated with diseased Canada thistle colonies in Montana, and
2. Examining their pathogenicity on Canada thistle, this research seeks to open new avenues in mycoherbicide research directed at Canada thistle control.

Materials and Methods

Pathogen Isolation

The Northern Frontier Organic Farms outside of Highwood Montana were previously reported as having thistle colonies displaying expanding circles of dead and dying thistle plants. During the summers of 2021 and 2022, pathogens were isolated from diseased Canada thistle colonies at this farm. In the year 2021, fungal isolations were done by Dr David Sands and group and were restricted to *Fusaria* species, whereas later in the year 2022 we conducted a more general survey of pathogens associated with dying Canada thistle was conducted. For 2022, root (including soil) and shoot samples were collected from the margin of dying thistle colonies at six

locations throughout a Canada thistle infested alfalfa field. From each field location, 3 samples of diseased roots and stems were collected in paper bags. These were immediately stored in a cooler with ice and brought back to the laboratory, where they were stored at 4° C until they could be processed within a five day period. For the pathogen isolation and observation, small 3mm sections of root/shoot samples were cut from the margins of healthy and diseased tissues, surface sterilized with 10% bleach solution for 30 seconds and rinsed with double distilled sterile water. For each plant sample, 5 tissue samples were then placed equidistance on a 120 mm petri dish containing ½ strength Potato Dextrose Agar with penicillin g (50 mg/l). These were sealed and incubated at room temperature (21°C) for five days. Plates were observed daily for emergent colony types and fungal colonies were identified based on morphological characters: hyphal growth, spore structures, colony color, etc. for five days post incubation.

Canada Thistle Stock Plants

Disease-free stock plants of Canada thistle were grown in a controlled isolation chamber in the Plant Growth Center, Montana State University. The temperatures were maintained at 25°C during the day and dropped to 22.2°C at night, with 16 hours photoperiod. Propagation of these plants was achieved through root cuttings equipped with viable root buds from a single healthy Canada thistle plant. Each root section was initially treated by dipping the cut section of root in a fungicidal solution of Stamina F4 at a concentration of 75ml Stamina F4 per liter of water, active ingredient concentrations: 0.638 grams of fluxapyroxad, 1.258 grams of pyraclostobin, 1.258 gram of triticonazole, 0.728 grams of metalaxyl per liter of water, (Bayer, New Jersey) to eliminate fungal infections and promote the development of healthy, disease-free plants. Subsequently, the treated cuttings were planted in round, 12.7 cm pots filled with a

homogeneous 50/50 soil mixture of Sunshine mix (soil-less mix is a blend of Canadian sphagnum peat moss and horticultural grade perlite) and MSU greenhouse soil mix (field soil, Canadian sphagnum peat moss, and washed concrete, sand are blended on a 1:1:1 by volume ratio). This approach was used to maintain health, and vigor of Canada thistle stock plants for greenhouse trials. The plants were watered every two days.

Fusaria Inoculum Preparation

Millet-based fungal inoculum was prepared by combining millet and water in a 2:1 ratio by volume. This was subsequently sterilized via autoclaving on the liquid cycle for 90 minutes in fungal culture bags (size: 8"×5"×19", Unicornbags, Plano, Texas, 75074) to eliminate any contaminating organisms within the media. Once cooled, subject *Fusaria* isolates were introduced into the sterile millet as agar plugs from pure cultures under aseptic conditions. Cultured bags were incubated until fully colonized, usually three weeks. The cultured millet was then dried in a laminar flow hood for two-four days i.e. until fully dry. In the case of the untreated control, the inoculated bags of *Fusarium* were re-sterilized after colony growth by autoclaving for one hour using a biohazard cycle, followed by the standard drying process. The dried inoculum and control was subsequently packaged in zip-lock bags and stored at 4 °C to maintain viability and prevent contamination.

Fusaria Pathogenicity Trials

Pathogenicity trials were conducted in controlled greenhouse in Montana State University Plant Growth Center located in Bozeman, Montana in the year 2023 and 2024. Trials used randomized complete block design using three different isolates of *Fusaria* – Bio-05, Bio-29 and Bio-82 with 4 replications and the trial was repeated once. The temperatures were maintained at

25°C during the day and dropped to 22.2°C at night, with 16 hours photoperiod. Treatments were implemented using 12.5 cm pots filled with a homogeneous 50/50 soil mixture of Sunshine mix and MSU mix (as previously described) and inoculated with 4 grams of the appropriate fungal inoculum or autoclaved inoculum for the un-inoculated control. The inoculum was thoroughly mixed into the upper 2 cm of soil to ensure even distribution. The next day, 25 mm section of healthy rootlet, displaying healthy root buds were excised and planted into the pots and watered. Pots were observed weekly for vigor and disease symptomology for seven weeks. Plant disease ratings were made weekly by using the un-inoculated control as the healthy plant reference with a rating of 0% diseased and a dead or dying plant receiving a rating of 100% diseased. At 7 weeks of post planting, all pots were harvested and observed for symptomology. Small root sections were cut from diseased tissue when present and cultured on half strength PDA in a petri dish. Five days later, the petri dishes were observed for fungal colonies, which were identified based on colony types and morphological characters.

Characterization of *Fusaria*

For the species level identification of *Fusaria*, Internal Transcribed Spacer (ITS) analysis was conducted. For ITS sequencing, DNA from 100mg of mycelium for each culture was extracted using a modified Kawasaki DNA extraction protocol (Frey and Frey, 1995; Holterman et al., 2012). The ITS region was amplified using fungal universal ITS primers, ITS1 Primer 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 Primer: 5'-TCCTCCGCTTATTGATATGC-3' (Cheng et al., 2016) and a standard PCR protocol (Ferrer et al., 2001). The PCR cycle included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 55°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C for 6 minutes. The PCR

products were observed on an agarose gel to verify successful amplification. The DNA samples were then sent for sequencing to GENEWIZ. The resultant sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) platform to identify *Fusarium* species.

Nematode Sampling 2024

During the summer of 2024, Canada thistle roots and surrounding soil were sampled for nematodes from the Northern Frontier Organic Farms. Five diseased root samples and five healthy root samples were collected, along with approximately 400 cc of soil from the surrounding rhizosphere. These samples were then transported to the laboratory for processing. Nematodes were extracted from the soil using the wet sieving method (Whitehead and Hemming, 1965). Each sample was spread on a Kimwipe laboratory tissue (30 × 30 cm; Kimtech), which was placed over a plastic 1mm mesh screen suspended on a metal frame at the water surface in plastic trays.

Extractions were conducted over a period of 48 hours at a temperature of 21°C. After extraction, the nematodes were concentrated by passing the nematode suspension through a 20-µm mesh sieve, followed by rinsing the trapped nematodes into 50-ml Falcon tubes using tap water. Resulting nematode suspensions were observed under a light microscope to determine species and concentrations. Roots tissues were stained to visualize the presence of nematodes within Canada thistle roots using acid fuchsin stain solution (Bybd et al., 1983). For this, roots were collected and thoroughly washed with tap water to remove soil and debris. After cleaning, the roots were fixed in FAA (Formalin-Acetic Acid-Alcohol) solution for 24 hours at room temperature. Following fixation, the roots were rinsed with distilled water and then cleared by

soaking them in 10% sodium hypochlorite (bleach) solution until they became partially transparent, approximately five minutes. The cleared roots were rinsed again with distilled water to remove any residual bleach. The roots were then stained with fuchsin acid solution (1 g of acid fuchsin in 100 ml of lactoglycerol, equal parts lactic acid, glycerol, and water). The roots were heated in the stain solution at 90°C for 5-10 minutes. After staining, the roots were destained in a lactoglycerol solution for several hours to allow for easy observation of stained nematodes. Stained roots were observed under a light microscope for the presence of nematodes and respective species.

Data Analysis

For our pathogenicity greenhouse trials, violation of assumption of ANOVA were observed from the residual vs fitted plot. So, a non-parametric test was performed. We used Kruskal-Wallis rank sum test to compare the mean pathogenicity across the treatments. A significant result from the Kruskal-Wallis rank sum indicated that there were differences in virulence between at least two of the treatments. To determine which specific isolates differed, a Pairwise comparison was performed using Wilcoxon rank sum test with continuity correction. This test allowed for pairwise comparisons of the mean pathogenicity scores between all isolate groups, adjusting for multiple comparisons to control for Type I error. The significance level was set at $\alpha < 0.05$ for all tests. To visualize the data, we used ggplot2 package. All statistical analyses were conducted using R statistical software version 4.2.2. For the identification of *Fusarium* species, the nucleotide sequences were blasted against NCBI database.

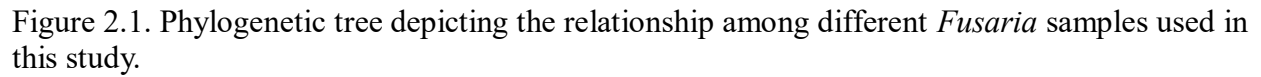
Results

Pathogen Isolation and Characterization

Eleven different *Fusaria* isolates collected from dying thistles in the year 2021, were tested and identified by ITS analysis. The ITS regions of the *Fusaria* isolates were compared with those reported in the database of National Center for Biotechnology Information (NCBI). According to the blast, the species level identification of the *Fusaria* showed that there were two species of *Fusarium oxysporum* and *Fusarium redolens* (Table 2.1.). Similarly, out of the 51 organisms that were isolated during the summer of 2022, we discovered that *Alternaria* species accounted for 68.4% of the isolates, *Fusaria* species for 25.2%, and some minor saprophytes including *Aspergillus*, *Penicillium*, and *Rhizopus* for the remaining 6.4% (Figure 2.2.). All *Fusaria* colony types and morphologies were consistent *F. oxysporum* or *F. redolens*. Soil and root sampling conducted in spring 2024 revealed the presence of the different nematodes like the Plant Parasitic Nematode, Root Lesion Nematode (RLN) *Pratylenchus neglectus*, bacterivorous, and fungivorous nematodes from the rhizosphere of Canada thistle from the Highwood location (Table 2.2.).

Table 2.1. Identification of *Fusaria* to species using ITS sequence analysis for eleven *Fusaria* isolates

S.no	Isolates	Closest homology	Identification success	Second closest homology	Identification success
1.	Bio-02	<i>Fusarium oxysporum</i>	100%	<i>Fusarium nirenbergiae</i>	100%
2.	Bio-04	<i>Fusarium redolens</i>	99.8%	<i>Fusarium hostae</i>	99.8%
3.	Bio-05	<i>Fusarium oxysporum</i>	100%	<i>Fusarium circinatum</i>	100%
4.	Bio-15	<i>Fusarium oxysporum</i>	99.2%	<i>Fusarium proliferatum</i>	99.2%
5.	Bio-17a	<i>Fusarium redolens</i>	100%	<i>Fusarium hostae</i>	100%
6.	Bio-22	<i>Fusarium oxysporum</i>	99.2%	<i>Fusarium nirenbergiae</i>	99.2%
7.	Bio-24	<i>Fusarium redolens</i>	100%	<i>Fusarium hostae</i>	100%
8.	Bio-29	<i>Fusarium redolens</i>	99.8%	<i>Fusarium hostae</i>	100%
9.	Bio42	<i>Fusarium oxysporum</i>	99.8%	<i>Fusarium circinatum</i>	99.8%
10.	Bio-46	<i>Fusarium oxysporum</i>	100%	<i>Fusarium nirenbergiae</i>	100%
11.	Bio-82	<i>Fusarium redolens</i>	99.8%	<i>Fusarium hostae</i>	99.8%



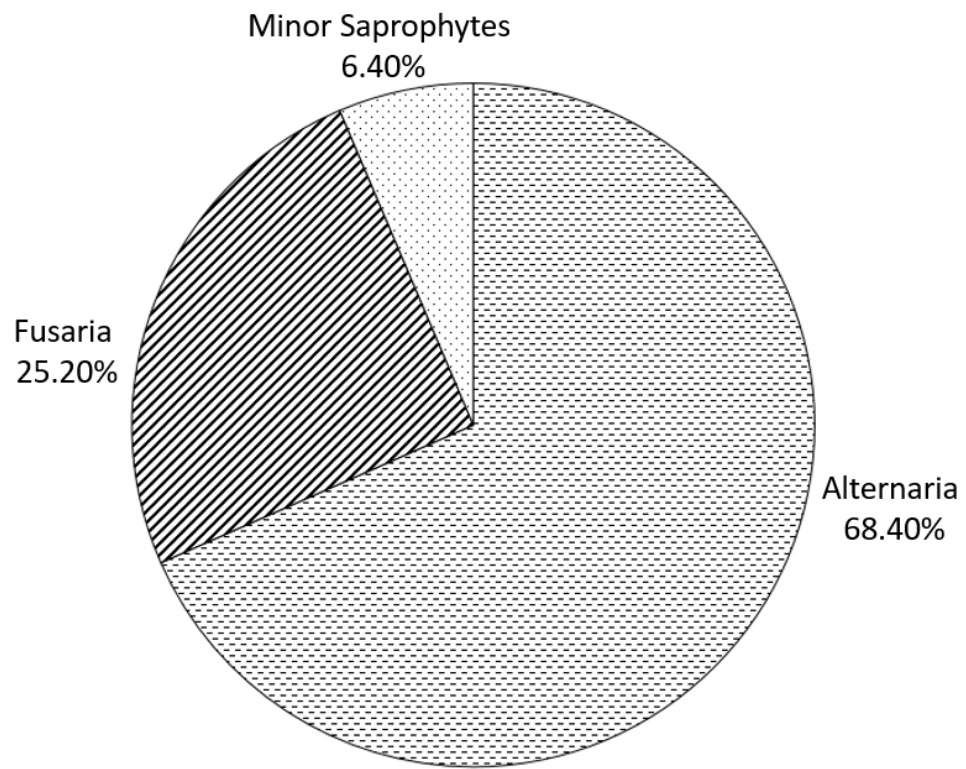


Figure 2.2. Results of fungal isolations from Canada thistle roots during the summer of 2022 from Northern Frontier Farms, Highwood, Montana.

Table 2.2. Different types of nematodes sampled from the rhizosphere of Canada thistle in the year 2024 from Northern Frontier Farm Highwood, Montana

Sample	Wet soil weight	RLN/Kg soil	Fungivores/Kg soil	Bacterivores/Kg soil	other PPN/Kg soil
1	83.49	4791	4192.1	5988.7	898.3
2	366.2	5393.2	204.8	614.4	68.3
3	121.2	4125.4	1443.9	2475.2	7425.7
4	131.2	1524.4	2096	762.2	2858.2
5	86.4	6076.4	5208.3	1157.4	1157.4
6	213.41	2928.6	1757.2	702.9	1640
7	323.9	4553.9	1003.4	1003.4	1775.2
8	257.1	3403.3	97.2	777.9	486.2
9	482.8	155.3	258.9	1087.4	2019.5
10	423.3	1240.3	590.6	354.4	767.8

Pathogenicity Test of *Fusarium*

The isolates used for this trial were one *Fusarium oxysporum* isolate (Bio-05) and two *Fusarium redolens* (Bio-29 and Bio-82). All of the three selected isolates of *Fusarium* were highly pathogenic in Canada thistle (Kruskal-Wallis $\chi^2 = 20.92$, $p < 0.001$) compared to the uninoculated controls (Fig 2.3.). Bio-05 was the most virulent isolate followed by Bio-82 and Bio-29. The pathogenicity scores, which is the measure of necrosis in Canada thistle for Bio-05, Bio-82 and Bio-29 were 99.3%, 92.5% and 83.1% respectively compared to the untreated controls with a pathogenicity scores of 20.8%. Isolation from root section from the diseased plants on half strength PDA resulted in re-isolation of *Fusarium* colony types and spore types were consistent with the respective species used in the inoculations (Fig 2.4.). No *Fusaria* were recovered from the untreated controls. Disease onset and symptom progression were noted in the treatment groups, while all the control plants remained free of symptoms and did not exhibit any signs of the disease, the diseased plants displayed symptoms such as stunted growth, yellowing of leaves, and root rot (Fig 2.4).

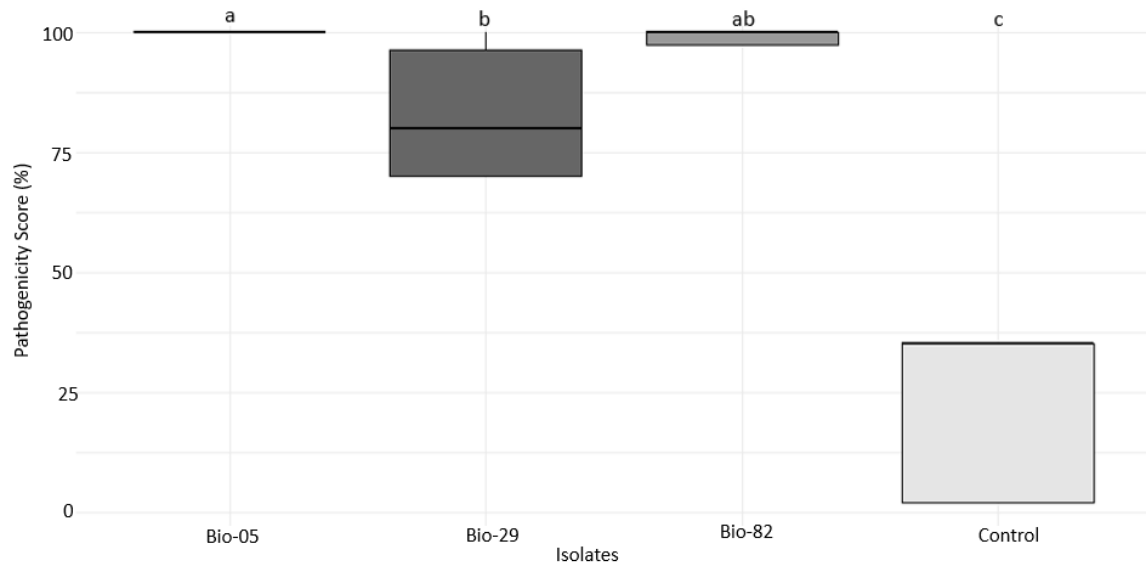


Figure 2.3. Average pathogenicity scores in Canada thistle when inoculated with treatments Bio-05 (*Fusaria oxysporum*), Bio-29 (*Fusarium redolens*) and Bio-82 (*Fusarium redolens*) compared to untreated control

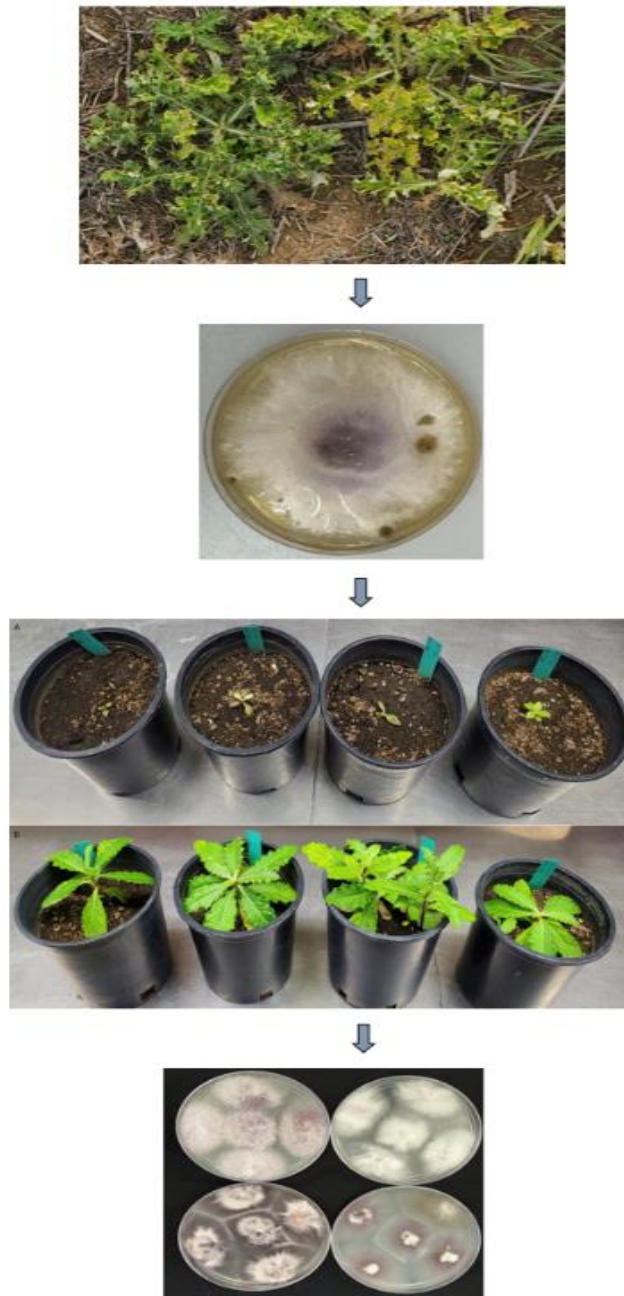


Figure 2.4. Fulfillment of Koch's Postulates for *Fusarium* Isolates on Canada Thistle. *Fusarium* isolated from diseased Canada thistle plants were tested on greenhouse-grown Canada thistle. Disease symptoms appeared in *Fusarium*-treated plants but were absent in untreated controls. Re-isolation of *Fusarium* from inoculated plants confirmed it as the causal agent of the disease in Canada thistle.

Discussion

The limited number of fungal species isolated from diseased Canada thistle (*Cirsium arvense*) suggests simple pathological interaction with limited pathogens species. These included unknown *Alternaria* species and two *Fusaria* species that are likely pathogens. All other fungi were likely saprophytes.

The *Fusaria* species accounted for a significant portion of the fungi isolated and are of particular interest due to their aggressive pathogenicity. The identification of predominately *F. oxysporum* and *F. redolens* based on morphologies, colony types and ITS sequencing were of particular interest. The former species is an aggressive wilt pathogen with typically narrow host range and has drawn particular interest as potential biocontrols for numerous weeds (Kohlschmid et al., 2009; Tiourebaev et al., 2010; Nazer Kakhaki et al., 2017). The latter species is considered an important pulse pathogen. Both species caused substantial damage and necrosis to Canada thistle. Their re-isolations from inoculated greenhouse plants for multiple trials fulfilled Koch's postulates and confirms for the first time that they are Canada thistle pathogens. Given their aggressiveness on Canada thistle, they could be good candidates as mycoherbicides for controlling Canada thistle.

The *Alternaria* species isolated from diseased Canada thistle was a significant observation given its well-documented role as a common plant pathogen (Logrieco et al., 2009). In the field survey, *Alternaria* was commonly isolated from necrotic stem and leaf tissue of Canada thistle. In our study, however its virulence and aggressiveness on Canada thistle was not explored at this time. *Alternaria* are common foliar pathogens causing leaf spots and blights. They can also act opportunistically, thriving in weakened hosts affected by mechanical injury,

competition, or other pathogens (Thomma, 2003). The presence of the root lesion nematode, *Pratylenchus neglectus* was not surprising given the crop history of the field location. Its pathogenicity on Canada thistle was not explored at this time. Regardless of its pathogenicity, its presence has been associated with greater infectivity of fusarium species on wide array of hosts (Taheri, 1996; Thompson and Clewett, 2021; Özdemir et al., 2022)

To develop either *F. oxysporum* or *F. redolens* as a biocontrol several issues must be addressed. The isolates must meet strict crop safety standards and also be safe to the environment. They must be able to effectively control Canada thistle under a variety of conditions. The virulence of *Fusarium* species may vary with environmental factors such as soil conditions, microbial interactions, temperature, and moisture levels (Shishido et al., 2005). Therefore, further research should focus on optimizing *Fusarium* use under variable agricultural conditions and determining how these fungi can be safely integrated into diverse ecosystems. Additionally, refinement may be needed to identify the most virulent *Fusarium* strains with the greatest biocontrol potential, understand timing, storability, optimal dosage and formulations.

In conclusion, this research identifies two new Canada thistle pathogens: *F. oxysporum* and *F. redolens*. Their ability to induce severe disease symptoms and reduce plant biomass makes them promising candidates for inclusion in sustainable weed management programs. However, before *Fusarium*-based biocontrol strategies can be widely adopted, further research is needed to overcome challenges related to environmental variability, host specificity, and field efficacy. Addressing these challenges could make *Fusarium*-based biocontrol methods among the most promising alternatives to chemical herbicides, contributing to sustainable agriculture and reduced reliance on synthetic chemicals.

References Cited

- Al-Khafaji, R. T., Gunnink Troth, E. E., Lambert, K. N., Johnston, J. A., & Dyer, A. T. (2019). Pathotypes detected among populations of *Pratylenchus neglectus* collected from Montana. *Plant disease*, 103(12), 3259-3264.
- Bybd Jr, D. W., Kirkpatrick, T., & Barker, K. (1983). An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of nematology*, 15(1), 142.
- Canada thistle biocontrol | Department of Agriculture. (n.d.). Retrieved December 5, 2024, from <https://ag.colorado.gov/conservation/biocontrol/canada-thistle>
- Chad W, Grekul., and Edward W, Bork. (2004). Herbage yield losses in perennial pasture due to Canada thistle (*Cirsium arvense*). *Weed Technology*, 18(3), 784-794.
<https://doi.org/10.1614/WT-03-196R>
- Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y., & Zhou, S. (2016). Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. *Molecular ecology resources*, 16(1), 138-149.
- Donald, W.W. (1994). Geostatistics for mapping weeds with a Canada thistle (*Cirsium arvense*) patch as a case study. *Weed Science*, 42, 648-657.
- Eberly, J.O., Hurd, A., Oli, D., Dyer, A.T., Seipel, T.F., and Carr, P.M. (2024). Compositional profiling of the rhizosphere microbiome of Canada thistle reveals consistent patterns across the United States northern Great Plains. *Sci Rep*, 14, 18016.
<https://doi.org/10.1038/s41598-024-69082-3>
- Enloe, S. F., Lym, R. G., Wilson, R., Westra, P., Nissen, S., Beck, G., and Halstvedt, M. (2007). Canada thistle (*Cirsium arvense*) control with aminopyralid in range, pasture and noncrop areas. *Weed Technology*, 21(4), 890–894. doi:10.1614/WT-07-004.1
- Ferrer, C., Colom, F., Frases, S., Mulet, E., Abad, J. L., & Alió, J. L. (2001). Detection and identification of fungal pathogens by PCR and by ITS2 and 5.8 S ribosomal DNA typing in ocular infections. *Journal of clinical microbiology*, 39(8), 2873-2879.
- French, R.C., and Lightfield. AR. (1990). Induction of systemic aecial infection in Canada thistle (*Cirsium arvense*) by teliospores of *Puccinia punctiformis*. *Phytopathology*, 80: 872–877. doi: 10.1094/Phyto-80-872
- Frey, J. E., & Frey, B. (1995). Molecular identification of six species of scale insects (*Quadraspidotus sp.*) by RAPD-PCR: assessing the field-specificity of pheromone traps. *Molecular ecology*, 4(6), 777-780.
- Halsted, B. D. (1894). Weeds and their most common fungi. New Jersey Exp. Stn. Rep, 1002.

- Heap, I. (2014). Global perspective of herbicide-resistant weeds. *Pest Manag. Sci.* 70, 1306–1315. doi:10.1002/ps.3696
- Hodgson, J.M. (1968). The nature, ecology, and control of Canada thistle. Technical Bulletin of the United States Department of Agriculture, No. 1386. United States Department of Agriculture, Washington, DC.
- Holterman, M. H., Oggenfuss, M., Frey, J. E., & Kiewnick, S. (2012). Evaluation of high-resolution melting curve analysis as a new tool for root-knot nematode diagnostics. *Journal of Phytopathology*, 160(2), 59-66.
- Jacobs, J., Sciegienka, J., & Menalled, F. D. (2006). Ecology and Management of Canada Thistle (*Cirsium arvense* (L.) Scop.). US Department of Agriculture, Natural Resources Conservation Service.
- Kluth, S., Kruess, A., and Tschardt, T. (2005). Effects of two pathogens on the performance of *Cirsium arvense* in a successional fallow. *Weed Research* 45: 261–269. doi:10.1111/j.1365-3180.2005.00463.x
- Kohlschmid, E., Sauerborn, J., & Müller-Stöver, D. (2009). Impact of *Fusarium oxysporum* on the holoparasitic weed *Phelipanche ramosa*: biocontrol efficacy under field-grown conditions. *Weed research*, 49, 56-65.
- Kurtz, Cassandra M. (2013). An Assessment of Invasive Plant Species Monitored by the Northern Research Station Forest Inventory and Analysis Program, 2005 through 2010. USDA, 43136. <https://doi.org/10.2737/nrs-gtr-109>
- Liebman, M., & Davis, A. S. (2009). Managing weeds in organic farming systems: an ecological approach. *Organic farming: the ecological system*, 54, 173-195.
- Logrieco, A., Moretti, A., & Solfrizzo, M. (2009). *Alternaria* toxins and plant diseases: an overview of origin, occurrence and risks. *World Mycotoxin Journal*, 2(2), 129-140.
- McLennan, B. R., et al. (1991). *Cirsium Arvense* (L.) Scop. Competition with Winter Wheat (*Triticum Aestivum* L.). *Weed Research*, 31(6), 409–415. <https://doi.org/10.1111/j.1365-3180.1991.tb01781.x>
- Miller, B. R., & Lym, R. G. (2017). Using the rosette technique for Canada thistle (*Cirsium arvense*) control in row crops. *Weed Technology*, 12(4), 699–706. doi:10.1017/S0890037X00044572
- Moyer, J. R., Schaalje, G. B., & Bergen, P. (1991). Alfalfa (*Medicago sativa*) seed yield loss due to Canada thistle (*Cirsium arvense*). *Weed Technology*, 5(4), 723-728.

- Müller, E., Jud, P., and Nentwig, W. (2011). Artificial infection of *Cirsium arvense* with the rust pathogen *Puccinia punctiformis* by imitation of natural spore-transfer of the weevil *Ceratapion onopordi*. *Weed Research*, doi: 10.1111/j.1365-3180.2010.00839.x
- Nazer, Kakhaki., S. H., et al. (2017). Biocontrol of Broomrape Using *Fusarium Oxysporum* F. *Sp. Orthoceras* in Tomato Crops under Field Conditions. *Biocontrol Science and Technology*, 27(12), 1435–1444, <https://doi.org/10.1080/09583157.2017.1409338>
- Orloff, N., Mangold, J., Miller, Z., & Menalled, F. (2018). A meta-analysis of field bindweed (*Convolvulus arvensis* L.) and Canada thistle (*Cirsium arvense* L.) management in organic agricultural systems. *Agriculture, Ecosystems & Environment*, 254, 264-272.
- O'Sullivan, P.A., Kossatz, V.C., Weiss, G.M., and Dew, D.A. (1982). An approach to estimating yield loss of barley due to Canada thistle. *Can J Plant Sci*, 62:125-131. <https://doi.org/10.4141/cjps82-105>
- O'Sullivan, P.A., Weiss, G.M., and Kossatz, V.C. (1985). Indices of competition for estimating rapeseed yield loss due to Canada thistle. *Can J Plant Sci* 65:145–149. <https://doi.org/10.4141/cjps85-020>
- Özdemir, F. G. G., Yaşar, B., Arıcı, Ş. E., & Elekcioglu, İ. H. (2022). Interaction effect of root lesion nematodes and *Fusarium culmorum* Sacc. on disease complex in some wheat cultivars. *Yuzuncu Yıl University Journal of Agricultural Sciences*, 32(1), 152-163.
- Pilipavičius, V., and Romaneckas, K. (2014). Allelopathic activity of creeping thistle water extracts on germination and early growth of winter wheat. *Bulgarian Journal of Agricultural Science* 20(3), 607–612 doi: 10.500.12259/87144
- Shishido, M., Miwa, C., Usami, T., Amemiya, Y., & Johnson, K. B. (2005). Biological control efficiency of *Fusarium* wilt of tomato by nonpathogenic *Fusarium oxysporum* Fo-B2 in different environments. *Phytopathology*, 95(9), 1072-1080.
- Taheri, A. (1996). Interaction between root lesion nematode, *Pratylenchus neglectus*, and root-rotting fungi of wheat (Doctoral dissertation).
- Thomma, B. P. (2003). *Alternaria spp.*: from general saprophyte to specific parasite. *Molecular plant pathology*, 4(4), 225-236.
- Thompson, J. P., & Clewett, T. G. (2021). Investigating the impact of root-lesion nematodes (*Pratylenchus thornei*) and crown rot (*Fusarium pseudograminearum*) on diverse cereal cultivars in a conservation farming system. *Agronomy*, 11(5), 867.
- Tiley, G.E. (2010). Biological flora of the British Isles: *Cirsium arvense* (L.) Scop. *J Ecol* 98(4):938–983. <https://doi.org/10.1111/j.1365-2745.2010.01678.x>

- Tiourebaev, K. S., Semenchenko, G. V., Dolgovskaya, M., McCarthy, M. K., Anderson, T. W., Carsten, L. D., Pilgeram, A.L., & Sands, D. C. (2001). Biological control of infestations of ditchweed (*Cannabis sativa*) with *Fusarium oxysporum f. sp. cannabis* in Kazakhstan. *Biocontrol science and technology*, 11(4), 535-540.
- Verbeek, J.D., and Kotanen, P.M. (2019). Soil-mediated impacts of an invasive thistle inhibit the recruitment of certain native plants. *Oecologia*, 190, 619–628.
<https://doi.org/10.1007/s00442-019-04435-8>
- Wandeler, H., and Bacher, S. (2006). Insect-transmitted urediniospores of the rust *Puccinia punctiformis* cause systemic infections in established *Cirsium arvense* plants. *Phytopathology* 96: 813–818. doi: 10.1094/PHYTO-96-0813
- Whitehead, A. G., & Hemming, J. R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of applied Biology*, 55(1), 25-38.
- Wilson, R. G. (1981). Effect of Canada thistle (*Cirsium arvense*) residue on growth of some crops. *Weed Science*, 29(2), 159-164.

CHAPTER THREE

EFFICACY AND CROP SAFETY ASSESSMENT OF FUSARIA
SPECIES

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Dipiza Oli

Contributions: Field planting, data and sample collection, data analysis, Writing-original draft

Co-Author: Alan T Dyer

Contributions: Resources, Conceptualization, Field Supervision, Data Validation, Review and editing manuscript

Manuscript Information

Dipiza Oli, Alan T Dyer

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Introduction

Canada thistle (*Cirsium arvense*) is an invasive perennial weed that causes significant damage to agricultural productivity and the stability of ecosystems (Stachon et al., 1980; Donald, 1994). Its vigorous growth habit, dense root system, and capacity for both sexual and asexual reproduction make it a formidable adversary in both cultivated and natural landscapes. In agricultural systems, Canada thistle competes aggressively for resources such as water, nutrients, and light, leading to significant yield reductions in important crops like wheat, beans, soybeans, and sugar beets (Beck, 2018). Additionally, the dense root system of Canada thistle can physically damage agricultural equipment and complicate field management practices (Ringselle et al., 2021). In natural ecosystems, its spread disrupts native plant communities, reducing biodiversity and altering habitat structures, making it a threat to both economic and ecological stability (Donald, 1994).

Control of Canada thistle is generally less of a problem for conventional growers than for those in organic production systems because of the wider array of herbicides and chemical treatments available in the market (Miller and Lym, 2017; Enloe et al., 2007). With conventional farming, synthetic herbicides, such as glyphosate, aminopyralid and 2,4-D, can be used effectively to suppress the growth of the undesired Canada thistle (Enloe, 2007; Miller and Lym, 2017). While chemical herbicides are effective solutions, the overreliance on these products has led to the development of herbicide-resistant weed populations of Canada thistle (Heap, 2014). Furthermore, the residual presence of synthetic herbicides in soil and water poses long-term environmental concerns, including contamination of water supplies and harm to non-target

organisms. As resistance builds and environmental regulations tighten, the need for sustainable and eco-friendly weed control methods becomes increasingly urgent (Kookana et al., 1998).

For organic farmers, weed management is often one of the most labor-intensive and costly aspects of production (Baker and Mohler, 2015). Crop cultivation and mechanical tillage are the common strategies for thistle control in organic system (Liebman and Davis, 2009; Orloff et al., 2018). These mechanical management however are not effective when they are applied alone and required a combination of two or more strategies such as crop competition, grazing, solarization, crop diversification and biocontrol (Orloff et al., 2018). In fact, cultivation and mowing does not effectively eliminate thistle as these practices fail to eradicate the root system. Instead, they may actually give rise to more thistle due to clonal expansion from small root fragments left behind after cutting (Edwards et al., 2000). Developing an effective biological control strategy could provide a vital tool for reducing reliance on manual weeding and mechanical methods, which can be both time-consuming and costly. Moreover, a biocontrol approach aligns with the principles of organic farming by promoting sustainability, preserving soil health, and minimizing the use of synthetic inputs.

Fusarium is one of the largest filamentous fungal genera with tens of thousands of species, some of which have been investigated for their potential as biocontrols of assorted weed species (Boyette et al., 1984; Pleban & Strobel, 1998; Tan et al., 2002; Vam Dam et al., 2018). In particular *Fusarium oxysporum* displays high host-specificity and, therefore, can target specific weed species without affecting non-target plants (Vam Dam et al., 2018). This reduces the risk of off-target effects toward crops and native vegetation species. *Fusarium oxysporum* is widely recognized for its capacity to infect specific hosts through root colonization, ultimately leading to

systemic infection and plant death. This mechanism provides a targeted approach to weed suppression, minimizing the risks posed to non-target species and surrounding flora. *Fusarium oxysporum* was initially screened for a mycoherbicide in Hawaii in the 1940s to determine its potential to reduce *Opuntia megacantha*, tree cactus (Fullaway, 1954; Wilson, 1969). Since then, *Fusarium oxysporum* spp has been shown in numerous studies to be effective candidates for the control of many weed species. Several formae speciales of *F. oxysporum* have been successfully deployed in biological control programs, such as *F. oxysporum* f.sp. *Cannabis* for controlling hemp (Tiourebaev, 2001; Punja, 2021), and *F. oxysporum* f.sp. *strigae* against striga (Venne et al., 2009; Lüth et.al., 2016). These examples underline the fungus's potential in developing targeted, eco-friendly weed management strategies. However, the success of any biocontrol strategy hinges on the agent's efficacy in suppressing the target weed and its safety concerning economically important crops.

This chapter aims to evaluate the efficacy of *F. oxysporum* as a biocontrol agent for Canada thistle and assess its impact on crop safety. By investigating the fungal isolate's efficacy and host specificity, and its impact on Canada thistle and other crop plants, this study seeks to advance our evaluation of its potential for use in organic and sustainable farming systems. Furthermore, these findings contribute to developing a biological control strategy that can reduce reliance on chemical inputs and support integrated weed management practices.

Materials and Methods

Canada Thistle Stock Plants

In the greenhouse, disease-free stock plants of Canada thistle were grown in a controlled isolation chamber. Propagation of these plants was achieved through root cuttings equipped with

viable root buds from a single healthy Canada thistle plant. Each root section was initially treated by dipping the cut section of root in a fungicidal solution of Stamina F4 at a concentration of 75ml Stamina F4 per liter of water, active ingredient concentrations: 0.638 grams of fluxapyroxad, 1.258 grams of pyraclostobin, 1.258 gram of triticonazole, 0.728 grams of metalaxyl per liter of water, (Bayer, New Jersey) to eliminate fungal infections and promote the development of healthy, disease-free plants. Subsequently, the treated cuttings were planted in round, 5-inch pots filled with a homogeneous 50/50 soil mixture of Sunshine mix (soil-less mix is a blend of Canadian sphagnum peat moss and horticultural grade perlite) and MSU greenhouse soil mix (field soil, Canadian sphagnum peat moss, and washed concrete, sand are blended on a 1:1:1 by volume ratio). This approach was used to maintain health, vigorous Canada thistle stock plants for research trials.

Fusaria Inoculum Preparation

Millet-based fungal inoculum was prepared by combining millet and water in a 2:1 ratio by volume. This was subsequently sterilized via autoclaving on the liquid cycle for 90 minutes in fungal culture bags (size: 8"×5"×19", Unicornbags, Plano, Texas, 75074) to eliminate any contaminating organisms within the media. Once cooled, subject *Fusaria* isolates were introduced into the sterile millet as agar plugs from pure cultures under aseptic conditions. Cultured bags were incubated until fully colonized, usually three weeks. The cultured millet was then dried in a laminar flow hood for two-four days i.e., until fully dry. In the case of the untreated control, the inoculated bags of *Fusarium* were re-sterilized after colonization by autoclaving for one hour using a biohazard cycle, followed by the standard drying process. The

dried inoculum and control were subsequently packaged in zip-lock bags and stored at 4 °C to maintain viability and prevent contamination.

Greenhouse Efficacy Trial

Efficacy trials were conducted at Montana State University Plant Growth Center in Bozeman, Montana in the year 2023 and 2024. Trials used randomized complete block design using eleven different isolates of *Fusaria* (Bio-02, Bio-04, Bio-05, Bio-15, Bio-17a, Bio-22, Bio-24, Bio-29, Bio-42, Bio-46, and Bio-82) with four replications for the first trial and six replications for the second trial. The temperatures were maintained at 25°C during the day and dropped to 22.2°C at night, with 16 hours photoperiod. Treatments were implemented using 12.5 cm pots filled with a 50/50 soil mixture of Sunshine mix and MSU mix (as previously described) and inoculated with 4 grams of the appropriate fungal inoculum or autoclaved inoculum for the un-inoculated control. The inoculum was thoroughly mixed into the upper 2 centimeters of soil to ensure even distribution. The next day, 25 mm sections of healthy rootlet displaying healthy root buds were excised and planted into the pots and watered. Pots were observed weekly for vigor and disease symptomology. Plant disease ratings were made weekly with completely healthy-looking thistle with a rating of 0% diseased and a dead or dying plant receiving a rating of 100% diseased. At 8 weeks post planting, the plants were observed for root rot and above ground portions were harvested to measure dry biomass of plants.

Efficacy Field Trial

In the summer of 2023 and 2024, field trials were conducted in Highwood and Big Timber, with local agricultural producers' cooperation. For the year 2023, trials were conducted in North Frontier Farms, Highwood and B-bar Ranch, Big Timber, Montana whereas for the year

2024, trials were set up in Highwood at North Frontier and Lassila Farms in Great Falls, Montana. The experimental design comprised five replicates of each treatment, an un-inoculated control and both single and combined inoculations in pairs with *Fusarium oxysporum* isolates Bio-02, Bio-05, and Bio-15, (total seven treatments) organized in a randomized complete block design for the year 2023 and an additional isolate, Bio-42 was added (total eight treatments) in the year 2024. Inoculum consisted of 300 grams of dried millet infested with the specified isolates. This was applied to plots measuring 1.8 meters by 3.05 meters, separated by 1.22 meters buffers to prevent cross-contamination using a spreader. For the co-inoculations, 150 grams of inoculum for each isolate was mixed and then applied using a spreader. Inoculations were conducted on May 16th at North Frontier Farms, Highwood and June 16th at Big Timber, respectively in the year 2023 and on May 14th and May 15th at North Frontier Farms and Lassila farm respectively for the year 2024. Data collection was done at the time of inoculation and again at the end of the growing season, focusing on thistle density, signs of thistle stem rot chlorosis, and percent regrowth.

Initial Crop Safety Greenhouse Trial

Two trials were initially conducted to examine crop safety of *Fusarium oxysporum* and *Fusarium redolens*. In the initial trials two isolates of *Fusarium oxysporum* (Bio-02 and Bio-15) and one isolate of *Fusarium redolens* (Bio-29) were used. In the second trial, one isolate of *Fusarium oxysporum* (Bio-05) and one isolate of *Fusarium redolens* (Bio-82) was chosen. These isolates were selected based on their efficacy on Canada thistle plants in greenhouse trials (data not shown). The greenhouse environmental conditions were the same as in the greenhouse efficacy trials, with the same soil composition and inoculum per pot. The trial used a randomized

complete block design with four replications. The crops tested against included wheat ('Duclair'), barley ('Craft'), lentil ('Avondale'), pea ('Montech'), chickpea ('Common Sierra'), safflower ('Local'), sunflower ('Local'), and Canada thistle as the positive control (First trial: eight plants and three isolates and a control= 32 treatments; Second trial: eight plants and two isolates and a control= 24 treatments). For Canada thistle, 25 mm of healthy rootlet with root buds of Canada thistle was plant in the inoculated pots. Data were taken on emergence for the first two weeks, and on weeks 1, 3, 5, and 7 plant vigor data were collected as a percentage on overall plant health. Eight weeks post planting, root rot data were taken, and above ground tissues were harvested, dried and weighed for measures of above ground plant biomass for each pot.

Crop Safety Greenhouse Trial

A more advanced crop safety trial was performed with multiple isolates of *Fusarium oxysporum* and *Fusarium redolens* to see if there is a generalized by the isolates of each species. Isolates were chosen based on preliminary greenhouse efficacy trials (data not shown). Isolates that displayed high levels of Canada thistle suppression were chosen for both the greenhouse and field crop safety trials. The greenhouse environmental conditions were the same as in the greenhouse efficacy trials, with the same soil composition and inoculum per pot. The trial used a randomized complete block design with six replications. For the first trial, treatments blocked by time with three replicates being applied on January 6, 2024, and the remaining three replicated on May 24, 2024. The crop safety trial used three isolates of *Fusarium oxysporum* group, Bio-02, Bio-05 and Bio-42; and three isolates of *Fusarium redolens*, Bio-04, Bio-29 and Bio-82; along with an untreated control. The crops tested against included wheat ('Duclair'), barley ('Craft'), lentil ('Avondale'), pea ('Montech'), chickpea ('Common Sierra'), safflower ('Local'), and Canada

thistle as the positive control (seven plants and six isolates and a control= 49 treatments). For Canada thistle, 25 mm of healthy rootlet with root buds of Canada thistle was plant in the inoculated pots. Data was taken on emergence for the first two weeks and on weeks 1, 3, 5, and 7 plant vigor data collected as a percentage on overall plant health. Eight weeks post planting, plant height were measure and above ground tissues were harvested, dried and weighed for measures of above ground plant biomass for each pot.

Crop Safety Field Trial

In the summer of 2023 and 2024, crop safety field trials were conducted at two environmentally distinct locations: the Lutz Research Farm 19.4 kilometers north of Bozeman, MT and Fort Ellis Research Farm 7.7 kilometers east of Bozeman, MT.

The trial included commonly grown crops in Montana which included wheat ('Duclair'), barley ('Craft'), lentil ('Avondale'), pea ('Montech'), chickpea ('Common Sierra'), and safflower ('Local').

Table 3.1. Planting specification for the crop safety trial

Crop	Variety	1000 seed weight(gram)	Seed rate per sq ft	seed/row (10 ft)	Weight (gram/row)
Wheat	Duclair	25.05	20	200	5.01
Barley	Craft	38.04	30	300	11.41
Lentil	Avondale	44.87	12	120	5.38
Chickpea	Common Sierra	39.84	4	40	15.62
Pea	Montech and Amarillo	21.64	8	80	17.31
Safflower	Local	31.84	20	200	6.37

The experimental design incorporated three isolates each from the *Fusarium oxysporum* group which are Bio-02, Bio-05, Bio-15 and the *Fusarium redolens* group which are Bio-04, Bio-29 and Bio-82, and untreated control with four replications (six crops and six isolates and a control= 42 treatments). In the year 2024, an additional isolate Bio-42 was added in the crop safety trial (49 treatments). Each plot consisted of four rows, 3 meters long. Each row received fifteen grams of millet-based inoculum, applied in furrow concurrently with planting. Data were collected on crop emergence, plant height, vigor, and yield. Three root samples were collected for each plot from two different replications to isolate fungal pathogens within the plant root systems. Small root sections were cut from diseased tissue when present and cultured on half strength PDA in a petri dish. Five days later, the petri dishes were observed for the presence of *Fusaria*.

Table 3.2. Planting and data collection dates

S.no	Task	2023	2024
1.	Planting	05/15/2023	05/18/2024
2.	Emergence	06/28/2023	06/04/2024
3.	Plant height and vigor	07/26/2023	07/12/2024
4.	Sampling	09/06/2023	06/13/2024
5.	Harvest	09/13/2023	09/09/2024

Data Analysis

For greenhouse efficacy trials, violation of assumption of ANOVA were observed from the residual vs fitted plot. So, a non-parametric test was performed instead of ANOVA. We used Kruskal-Wallis rank sum test to compare the mean pathogenicity across the treatments. A significant result from the Kruskal-Wallis rank sum indicated that there were differences in

virulence between at least two of the treatments. To determine which specific isolates differed, a Pairwise comparison was performed using Wilcoxon rank sum test with continuity correction.

A two-way ANOVA was carried out to analyze greenhouse crop safety trials and the field crop safety trials. To identify difference among treatments, a post-hoc analysis was performed utilizing Tukey's Honest Significant Difference (HSD) test for pairwise comparisons with significance level set at $\alpha < 0.05$. All statistical analyses were conducted using R statistical software version 4.2.2.

Results

Greenhouse Efficacy Trials

Overall, all the isolates of *Fusarium* caused disease in Canada thistle compared to the un-inoculated controls (Figure 3.1). The Kruskal-Wallis rank sum test results indicate that there were statistically significant differences in disease severity among the different *Fusarium* isolates ($\chi^2 = 34.85$, $p < 0.001$). The mean disease severity for *Fusarium oxysporum* isolates, *Fusarium redolens* isolates and the control were 37.8%, 40.8% and 4.7%, respectively (Table 3.3). *Fusarium redolens* isolate Bio-04 had the highest disease severity at 83% followed by *Fusarium oxysporum* isolate Bio-05 at 79% (Table 3.3).

Also, *Fusaria* treatment caused significant root rot ($\chi^2 = 29.735$, $p = 0.001$) with Bio-04 and Bio-05 with highest root rot severity scores at 59.4% and 60.5% respectively. The untreated control had a root rot rating of 0.8% (Table 3.3). Significant reduction in dry biomass were also observed ($\chi^2 = 29.83$, $p = 0.001$) in Canada thistle compared to the un-inoculated controls. Bio-05 had the lowest thistle dry biomass of 0.08 grams followed by Bio-04 of 0.10 grams, whereas the biomass of untreated controls of Canada thistle were 1.2 grams (Table 3.3).

Table 3.3. Average disease severity, root rot and dry biomass of Canada thistle for different isolates of *Fusarium* in greenhouse trials.

Isolates	Species	Mean disease severity	Mean Root Rot	Mean Biomass
Bio 02	<i>Fusarium oxysporum</i>	42.8±11.3	22.9 ± 14.6	1.2± 0.2
Bio 04	<i>Fusarium redolens</i>	83.3±7.2	59.4± 11.9	0.1±0.05
Bio 05	<i>Fusarium oxysporum</i>	79.0±10.7	60.6± 11.1	0.08±0.05
Bio 15	<i>Fusarium oxysporum</i>	14.4±10.8	12.9± 10.9	1.6±0.3
Bio 17a	<i>Fusarium redolens</i>	14.6±10.9	12.3± 11.0	1.5±0.2
Bio 22	<i>Fusarium oxysporum</i>	34.7± 15.2	27.3±14.4	0.9±0.3
Bio 24	<i>Fusarium redolens</i>	37.4±14.2	34.0± 16.5	1.2±0.3
Bio 29	<i>Fusarium redolens</i>	39.0±15.3	28.9± 14.0	0.6±0.2
Bio 42	<i>Fusarium oxysporum</i>	35.0±16.3	34.0±16.5	0.9±0.3
Bio 46	<i>Fusarium oxysporum</i>	21.4±10.0	5.1± 2.9	1.2±0.3
Bio 82	<i>Fusarium redolens</i>	30.1±10.3	14.0± 10.8	0.8±0.2
Control	Control	4.8 ± 3.8	0.7±0.15	1.3±0.1
p-value		0.0002	0.001	0.001
χ^2		34.85	29.73	29.83



Fig 3.1 Canada thistle plants on the seventh week of greenhouse efficacy trial inoculated with (top to bottom) *Fusarium oxysporum* isolate Bio-05, un-inoculated control and *Fusarium redolens* isolate Bio-04.

Field Efficacy Trial

The field efficacy trials conducted in North Frontier Farms Highwood in 2023 demonstrated that *Fusarium oxysporum* isolates significantly reduced Canada thistle stands (ANOVA, $p = 0.023$) where isolates Bio-02 and Bio-05 reduced thistle stands by 79.2% and 61.2%, respectively and the combination of Bio-05 and Bio-15 reducing stands by 73.9% (Figure 3.2). While not significant, a similar trend was observed was observed stem necrosis at Big Timber ($R^2 = 0.18$, $p = 0.001$). In 2024, no significant reduction in thistle stand was observed at either North Frontier Farm or Lasilla farm (ANOVA, $p = 0.20$ and $p = 0.97$ respectively).

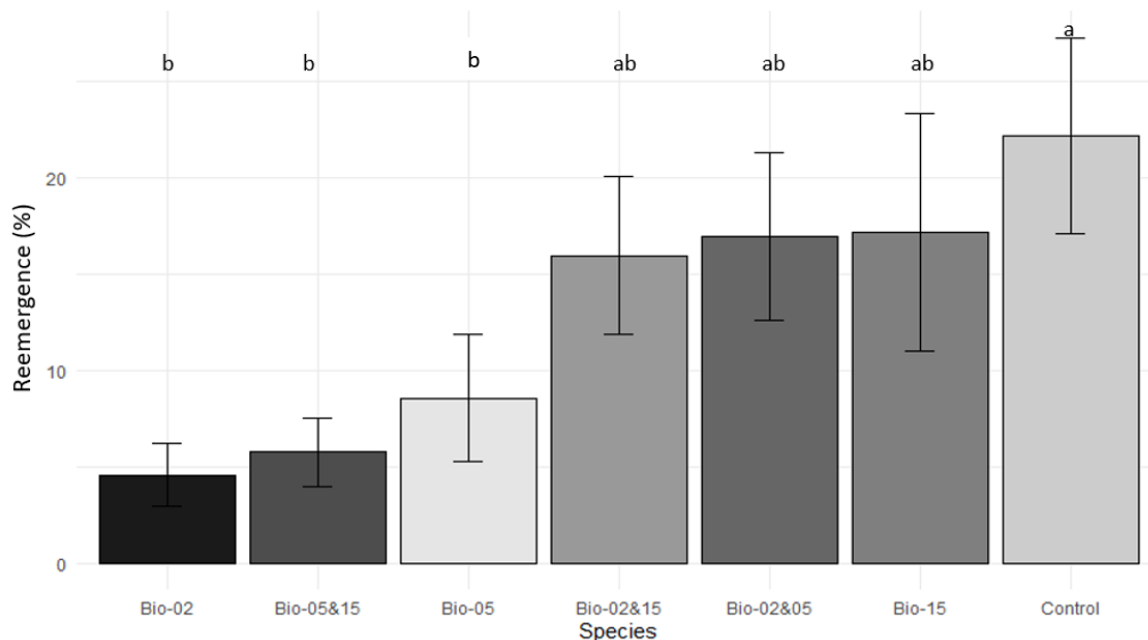


Figure 3.2. Reemergence of Canada thistle with application of different *Fusaria* isolates in the year 2023

Initial Crop Safety Trial

Inoculations for *Fusarium oxysporum* isolates (Bio-02, Bio-05, and Bio-15) had no adverse effects on wheat, barley, pea, lentil, chickpea, safflower, and sunflower (Figure 3.5.1 - Figure 3.5.8 and Table 3.4.1 - Table 3.4.8) in both trials. In contrast, relative to the negative controls, *Fusarium redolens* isolate Bio-29, and Bio-82 significantly reduced emergence of barley, peas and lentils and vigor of wheat, lentil, pea in trials 1 and 2 respectively (Kruskal-Wallis, $p < 0.05$). Bio 29 and Bio 82 also reduced biomass of wheat, lentil, (ANOVA, $p < 0.01$) and caused significant root rot in lentil (ANOVA, $p = 0.05$). Both *F. oxysporum* and *F. redolens* isolates negatively affected emergence, vigor, and dry weight of Canada thistle (ANOVA, $p < 0.05$). Bio-02 and Bio-15 had less effect on Canada thistle compared to Bio-05, Bio-29 and Bio-82.

Table 3.4.1. Emergence (%) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-05 and Bio-82 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error. Different letters within the same crop indicate significant differences between treatments (Kruskal-Wallis, $p < 0.05$).

Isolates	Emergence (%)							
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower	Sunflower	Canada thistle
Bio-05	87.5 $\pm$ 7.2 a	87.5 $\pm$ 7.2	68.8 $\pm$ 15.7 a	87.5 $\pm$ 7.2	62.5 $\pm$ 16.1	81.2 $\pm$ 6.2	87.5 $\pm$ 12.5	0.0 $\pm$ 0.0 b
Bio-82	25.0 $\pm$ 10.2 b	75.0 $\pm$ 10.2	43.8 $\pm$ 11.9 b	62.5 $\pm$ 16.1	18.8 $\pm$ 6.2	68.8 $\pm$ 6.2	68.8 $\pm$ 15.7	0.0 $\pm$ 0.0 b
Control	93.8 $\pm$ 6.2 a	93.8 $\pm$ 6.2	100.0 $\pm$ 0.0 a	93.8 $\pm$ 6.2	75.0 $\pm$ 17.7	93.8 $\pm$ 6.2	87.5 $\pm$ 7.2	100.0 $\pm$ 0.0 a
p-value	0.01	0.30	0.02	0.21	0.06	0.07	0.47	0.004
p-value	<0.01							

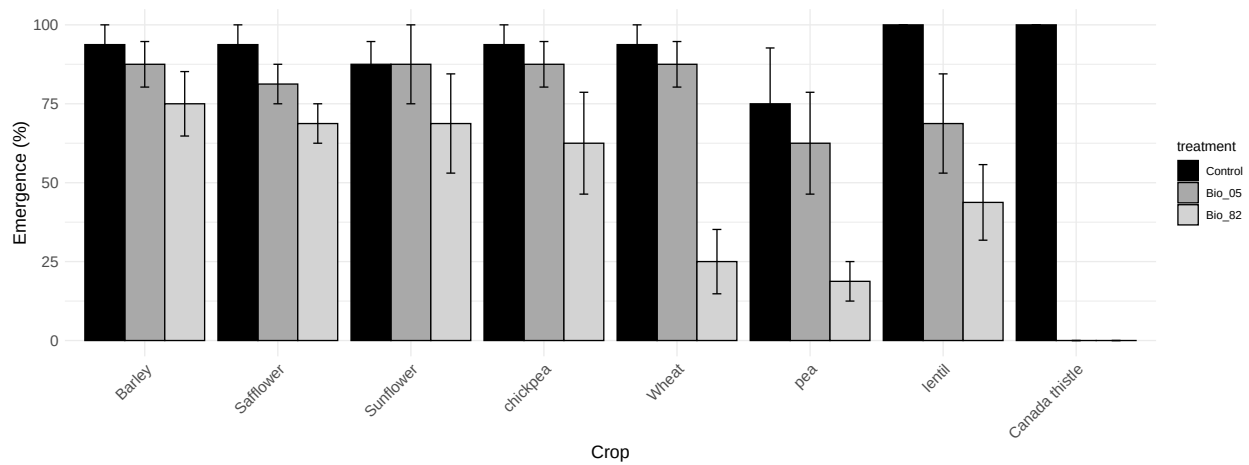


Figure 3.3.1. Emergence (%) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-05 and Bio-82 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error.

Table 3.4.2. Emergence (%) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-02, Bio-15 and Bio-29 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error. Different letters within the same crop indicate significant differences between treatments (Kruskal-Wallis, $p < 0.01$).

Isolates	Emergence (%)							
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower	Sunflower	Canada thistle
Bio-02	100.0 $\pm$ 0.0	93.8 $\pm$ 6.2 a	93.8 $\pm$ 6.2	100.0 $\pm$ 0.0 a	68.8 $\pm$ 12.0	100.0 $\pm$ 0.0	75.0 $\pm$ 10.2	0.0 $\pm$ 0.0
Bio-15	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0 a	87.5 $\pm$ 7.2	100.0 $\pm$ 0.0 b	81.2 $\pm$ 6.2	100.0 $\pm$ 0.0	81.2 $\pm$ 12.0	0.0 $\pm$ 0.0
Bio-29	87.5 $\pm$ 7.2	75.0 $\pm$ 0.0 b	56.2 $\pm$ 12.0	56.2 $\pm$ 12.0 a	50.0 $\pm$ 10.2	100.0 $\pm$ 0.0	87.5 $\pm$ 12.5	0.0 $\pm$ 0.0
Control	81.2 $\pm$ 6.2	100.0 $\pm$ 0.0 a	87.5 $\pm$ 7.2	100.0 $\pm$ 0.0 a	56.2 $\pm$ 12.0	87.5 $\pm$ 7.2	81.2 $\pm$ 12.0	0.0 $\pm$ 0.0
p-value	0.06	0.008	0.08	0.002	0.19	0.09	0.82	1.00
p-value	<0.01							

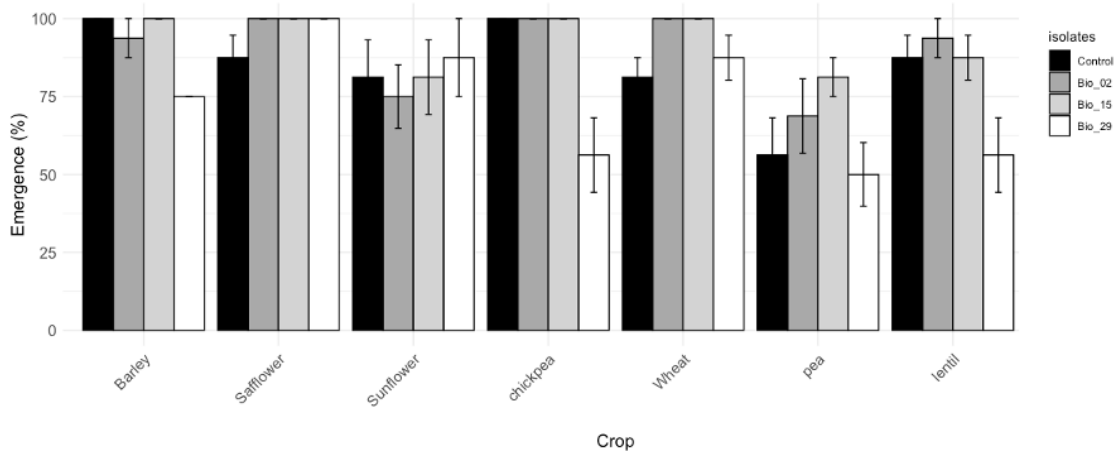


Figure 3.3.2. Emergence (%) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-02, Bio-15 and Bio-29 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error.

Table 3.4.3. Vigor (%) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-05 and Bio-82 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error. Different letters within the same crop indicate significant differences between treatments (Kruskal-Wallis, $p < 0.05$).

Isolates	Plant Vigor (%)							
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower	Sunflower	Canada thistle
Bio-05	70.0 $\pm$ 0.0 a	70.0 $\pm$ 0.0	23.0 $\pm$ 11.6 ab	53.2 $\pm$ 5.2	57.5 $\pm$ 12.5 a	62.5 $\pm$ 3.2	72.5 $\pm$ 1.4	0.0 $\pm$ 0.0 b
Bio-82	31.2 $\pm$ 18.3 b	68.7 $\pm$ 1.2	9.7 $\pm$ 6.8 b	49.5 $\pm$ 10.4	17.0 $\pm$ 15.4 b	63.7 $\pm$ 1.2	73.7 $\pm$ 1.2	0.0 $\pm$ 0.0 b
Control	70.0 $\pm$ 0.0 a	70.0 $\pm$ 0.0	53.7 $\pm$ 5.5 a	63.2 $\pm$ 3.5	69.5 $\pm$ 2.10 a	66.2 $\pm$ 2.4	75.7 $\pm$ 0.7	78.7 $\pm$ 1.2 a
p-value	0.02	0.37	0.03	0.35	0.03	0.62	0.56	0.02
p-value	<0.01							

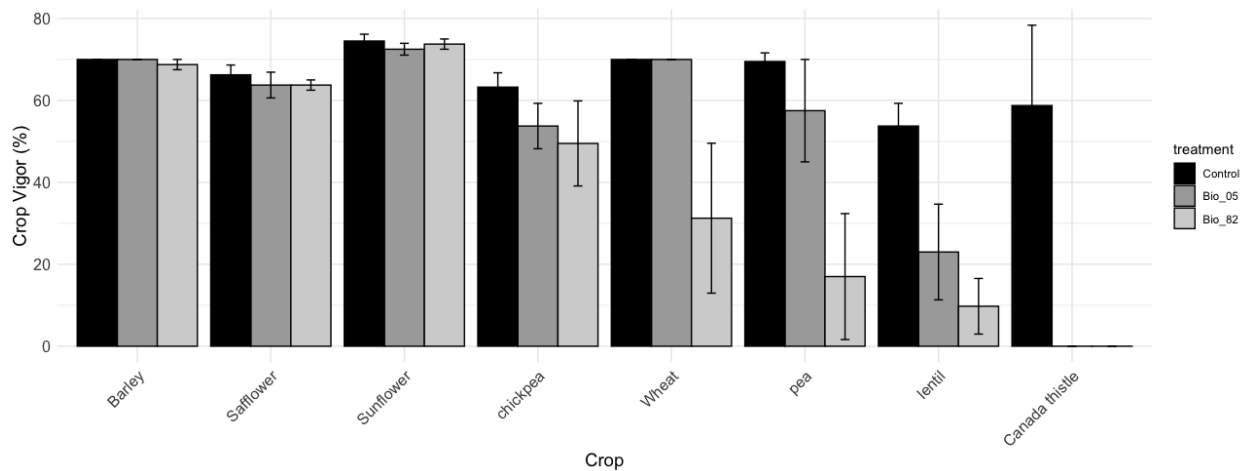


Figure 3.3.3. Vigor (%) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-05 and Bio-82 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error.

Table 3.4.4. Vigor (%) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-02, Bio-15 and Bio-29 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error. Different letters within the same crop indicate significant differences between treatments (Kruskal-Wallis, $p < 0.05$).

Isolates	Plant Vigor (%)							
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower	Sunflower	Canada thistle
Bio-02	69.2 $\pm$ 1.4	70.2 $\pm$ 0.2	69.5 $\pm$ 1.0	70.2 $\pm$ 0.9	80.5 $\pm$ 0.5	69.0 $\pm$ 1.0	71.5 $\pm$ 2.2	63.8 $\pm$ 1.2 a
Bio-15	70.0 $\pm$ 0.0	68.2 $\pm$ 1.2	68.0 $\pm$ 1.2	71.5 $\pm$ 0.5	79.0 $\pm$ 0.7	70.0 $\pm$ 0.0	72.2 $\pm$ 1.7	56.2 $\pm$ 10.5 a
Bio-29	70.0 $\pm$ 0.0	67.5 $\pm$ 1.0	58.5 $\pm$ 9.5	68.8 $\pm$ 0.7	77.5 $\pm$ 1.0	68.5 $\pm$ 1.0	74.2 $\pm$ 1.9	6.2 $\pm$ 1.2 b
Control	70.0 $\pm$ 0.0	68.8 $\pm$ 1.2	69.5 $\pm$ 0.5	73.5 $\pm$ 2.2	75.0 $\pm$ 3.4	68.5 $\pm$ 0.5	71.8 $\pm$ 1.2	70.0 $\pm$ 2.0 a
p-value	0.81	0.16	0.35	0.06	0.18	0.31	0.91	0.009
p-value	<0.01							

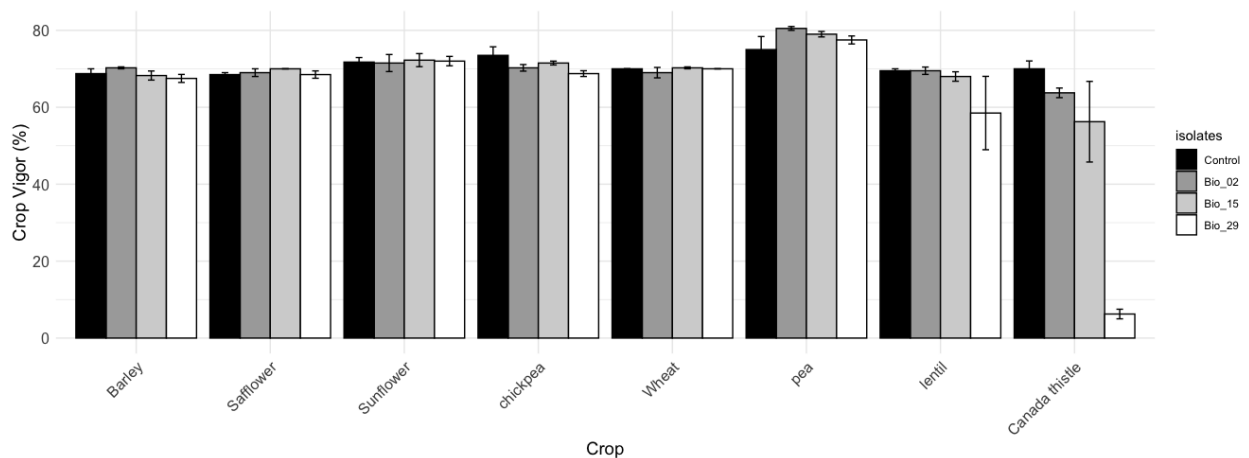


Figure 3.3.4. Vigor (%) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-02, Bio-15, and Bio-29 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error.

Table 3.4.5. Average dry biomass (grams) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-02, Bio-15 and Bio-29 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error. Different letters within the same crop indicate significant differences between treatments (Kruskal-Wallis, $p < 0.05$).

Isolates	Biomass (grams)							
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower	Sunflower	Canada thistle
Bio-05	19.8 $\pm$ 1.2 a	25.82 $\pm$ 1.2	10.4 $\pm$ 0.2 b	15.2 $\pm$ 1.7	14.32 $\pm$ 1.4 a	12.35 $\pm$ 0.62	32.6 $\pm$ 1.9	0.0 $\pm$ 0.0 b
Bio-82	6.92 $\pm$ 4.1 b	22.9 $\pm$ 1.9	7.7 $\pm$ 2.3 b	13.6 $\pm$ 1.3	7.85 $\pm$ 2.64 b	11.85 $\pm$ 0.22	31.57 $\pm$ 1.9	0.0 $\pm$ 0.0 b
Control	20.5 $\pm$ 0.6 a	26.45 $\pm$ 0.3	13.7 $\pm$ 1.2 a	18.9 $\pm$ 1.8	19.17 $\pm$ 2.4 a	14.42 $\pm$ 1.0	34.12 $\pm$ 0.9	11.9 $\pm$ 0.2 a
p-value	0.02	0.33	0.01	0.12	0.03	0.06	0.78	0.02
p-value	<0.01							

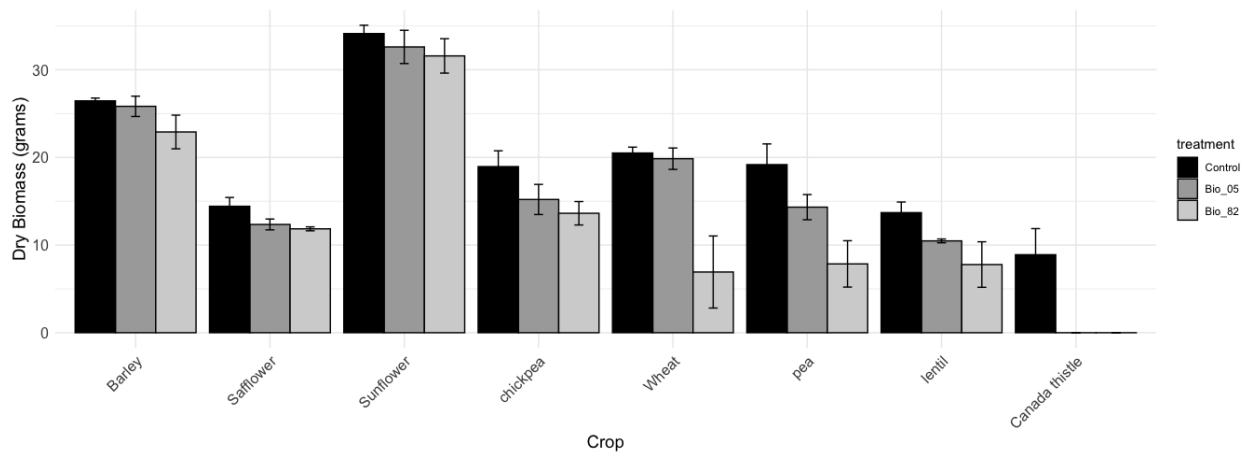


Figure 3.3.5. Average dry biomass (grams) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-05, and Bio-82 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error.

Table 3.4.6. Average dry biomass (grams) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-02, Bio-15 and Bio-29 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error. Different letters within the same crop indicate significant differences between treatments (Kruskal-Wallis, $p < 0.05$).

Isolates	Biomass (grams)							
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower	Sunflower	Canada thistle
Bio-02	10.9 $\pm$ 0.5	15.9 $\pm$ 1.7	8.0 $\pm$ 2.5	12.3 $\pm$ 2.2	25.0 $\pm$ 3.4	7.8 $\pm$ 0.5	24.8 $\pm$ 3.5	1.6 $\pm$ 0.5 a
Bio-15	11.2 $\pm$ 1.8	10.0 $\pm$ 1.4	7.3 $\pm$ 2.4	12.4 $\pm$ 2.9	22.4 $\pm$ 4.5	7.1 $\pm$ 0.6	24.1 $\pm$ 1.9	2.9 $\pm$ 0.5 a
Bio-29	9.6 $\pm$ 1.1	10.6 $\pm$ 2.1	4.1 $\pm$ 1.3	6.7 $\pm$ 1.6	13.5 $\pm$ 3.7	7.4 $\pm$ 1.1	27.0 $\pm$ 3.1	0.1 $\pm$ 0.1 b
Control	11.7 $\pm$ 2.1	10.5 $\pm$ 0.7	7.9 $\pm$ 0.8	10.0 $\pm$ 0.8	18.8 $\pm$ 5.8	7.8 $\pm$ 1.0	26.7 $\pm$ 2.8	1.3 $\pm$ 0.4 a
p-value	0.65	0.11	0.29	0.24	0.31	0.94	0.73	0.01
p-value	<0.01							

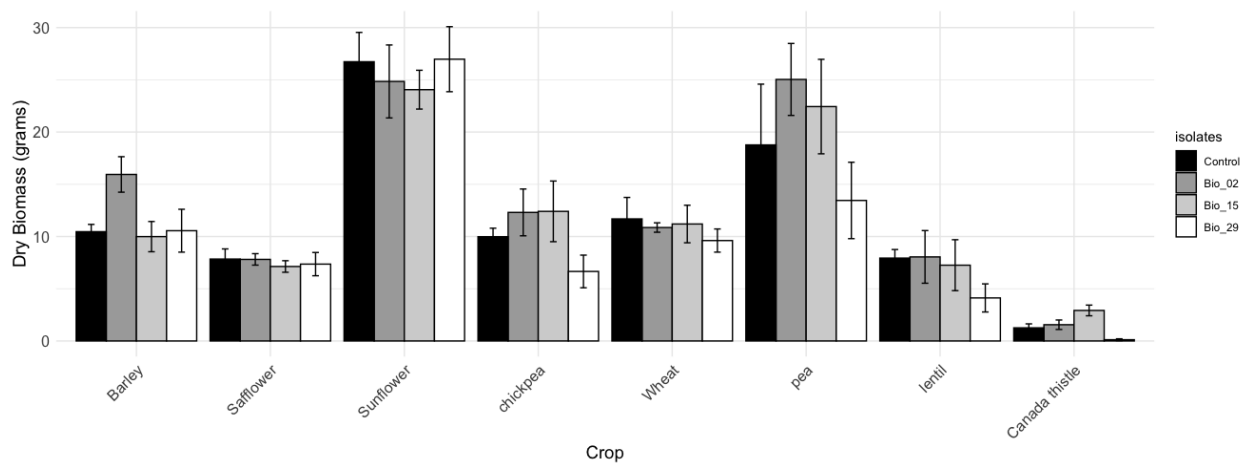


Figure 3.3.6. Average dry biomass (grams) of various crops and Canada thistle inoculated with *Fusarium* isolates Bio-02, Bio-15, and Bio-29 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error.

Table 3.4.7. Root rot (%) in various crops and Canada thistle inoculated with *Fusarium* isolates Bio-05, and Bio-82 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error. Different letters within the same crop indicate significant differences between treatments (Kruskal-Wallis, $p < 0.05$).

Isolates	Root rot (%)							
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower	Sunflower	Canada thistle
Bio-05	2.5 $\pm$ 1.4	9.0 $\pm$ 1.5	65.5 $\pm$ 16.0 ab	43.2 $\pm$ 12.9	47.0 $\pm$ 22.2	27.5 $\pm$ 15.1	0.00 $\pm$ 0.0	100.0 $\pm$ 0.0 b
Bio-82	50.0 $\pm$ 28.8	9.2 $\pm$ 5.6	76.2 $\pm$ 17.3 b	40.0 $\pm$ 13.4	80.0 $\pm$ 12.2	22.5 $\pm$ 5.9	0.00 $\pm$ 0.0	100.0 $\pm$ 0.0 b
Control	0.00 $\pm$ 0.0	0.7 $\pm$ 0.7	20.0 $\pm$ 7.35 a	18.7 $\pm$ 5.5	40.0 $\pm$ 10.0	11.2 $\pm$ 3.7	0.00 $\pm$ 0.0	0.00 $\pm$ 0.0 a
p-value	0.24	0.13	0.05	0.16	0.10	0.51	1.00	0.02
p-value	<0.01							

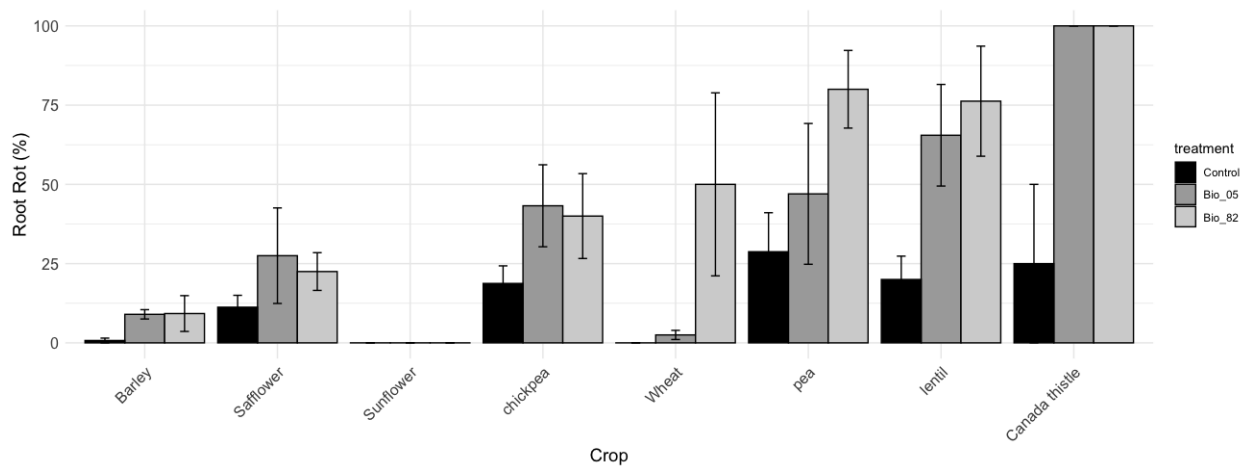


Figure 3.3.7. Root rot (%) in various crops and Canada thistle inoculated with *Fusarium* isolates Bio-05, and Bio-82 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error.

Table 3.4.8. Root rot (%) in various crops and Canada thistle inoculated with *Fusarium* isolates Bio-02, Bio-15 and Bio-29 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error. Different letters within the same crop indicate significant differences between treatments (Kruskal-Wallis, $p < 0.05$).

Isolates	Root rot							
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower	Sunflower	Canada thistle
Bio-02	9.2 $\pm$ 2.5	17.5 $\pm$ 1.4	3.0 $\pm$ 1.2	5.5 $\pm$ 3.4	8.8 $\pm$ 3.0	1.5 $\pm$ 1.0	0.0 $\pm$ 0.0	4.2 $\pm$ 2.5 a
Bio-15	9.0 $\pm$ 2.7	13.5 $\pm$ 4.0	9.5 $\pm$ 3.6	15.0 $\pm$ 2.1	6.2 $\pm$ 2.2	4.5 $\pm$ 2.6	0.0 $\pm$ 0.0	18.2 $\pm$ 17.3 a
Bio-29	10.5 $\pm$ 0.9	30.0 $\pm$ 3.5	15.2 $\pm$ 5.0	10.8 $\pm$ 5.7	9.8 $\pm$ 5.7	1.8 $\pm$ 1.8	0.0 $\pm$ 0.0	78.8 $\pm$ 18.1 b
Control	7.5 $\pm$ 3.1	25.0 $\pm$ 6.1	6.8 $\pm$ 2.7	14.0 $\pm$ 5.3	10.8 $\pm$ 5.2	1.2 $\pm$ 1.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0 b
p-value	0.87	0.09	0.13	0.46	0.79	0.70	1.00	0.02
p-value	<0.01							

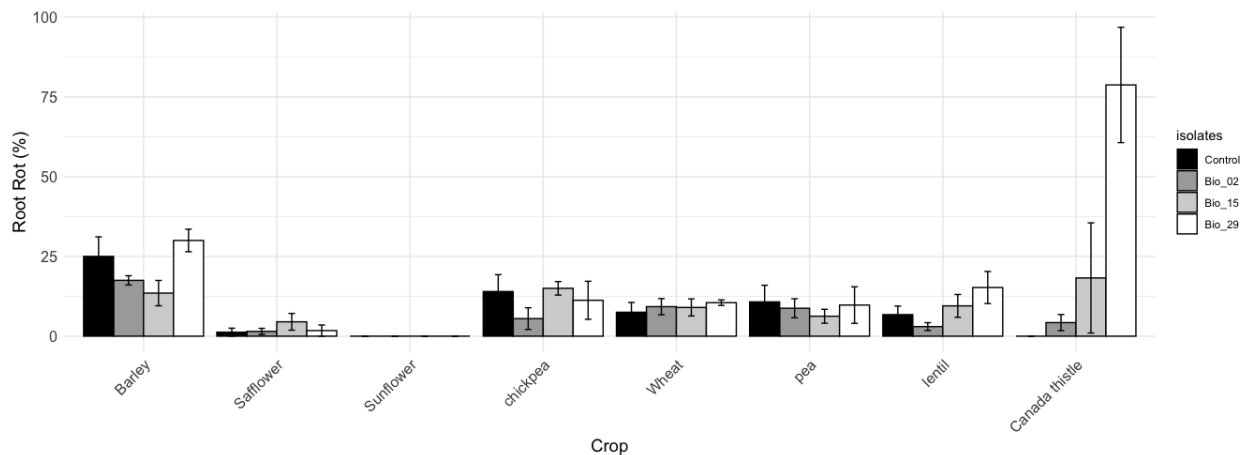


Figure 3.3.8. Root rot (%) in various crops and Canada thistle inoculated with *Fusarium* isolates Bio-05, and Bio-82 compared to the un-inoculated control. Values are presented as mean $\pm$ standard error.

Extended Crop Safety Trial

Emergence rates for wheat, barley, lentil, chickpea, pea, and safflower were $88.8 \pm 5.2\%$, $88.8 \pm 4.6\%$, $82.4 \pm 7.3\%$, $62.0 \pm 8.0\%$, $64.8 \pm 8.3\%$ and $83.8 \pm 7.0\%$ respectively across the different *Fusarium* isolates (Table 3.5). For comparison un-inoculated control (UTC) had emergence rates of $94.4 \pm 3.7\%$, $91.6 \pm 4.3\%$, $94.4 \pm 5.5\%$, $75.0 \pm 7.2\%$, $66.6 \pm 5.8\%$, and $86.11 \pm 6\%$ for wheat, barley, lentil, chickpea, pea, and safflower (Table 3.5). A significant difference in emergence was observed among different crops (ANOVA, $p < 0.01$). However, no significant difference was observed among isolates for any crop or between them and the un-inoculated control (ANOVA, $p > 0.05$). Canada thistle served as the positive control in these trials. In contrast to the crop results, *Fusarium* isolates significantly inhibited the emergence of Canada thistle (ANOVA, $p = 0.006$), with emergence values of $0.0 \pm 0.0\%$, $0.0 \pm 0.0\%$, $8.3 \pm 8.3\%$ for isolates Bio-05, Bio-82 and Bio-04. Bio-02, Bio-29, and Bio-42 had significantly higher emergence values of 16.7 ± 11.2 and 16.7 ± 11.2 , respectively. The un-inoculated control had the highest emergence rate for Canada thistle ($50.00 \pm 15.08\%$) (Table 3.5).

Isolates	Plant emergence (%)						
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower	Canada thistle
Bio-02	91.6 ± 4.3	91.6 ± 5.9	86.1 ± 4.9	69.4 ± 8.6	63.9 ± 8.6	94.4 ± 5.5	16.6 ± 11.2 b
Bio-04	86.1 ± 6.4	83.3 ± 6.4	80.5 ± 6.4	55.5 ± 7.5	63.9 ± 7.6	83.3 ± 6.5	8.3 ± 8.3 b
Bio-05	97.2 ± 2.7	83.3 ± 5.0	86.1 ± 6.4	66.6 ± 7.1	80.5 ± 7.6	86.1 ± 4.9	0.0 ± 0.0 b
Bio-29	94.4 ± 3.7	80.5 ± 6.4	83.3 ± 8.7	66.6 ± 7.1	63.9 ± 10.4	80.5 ± 8.6	16.6 ± 11.2 b
Bio-42	83.3 ± 6.4	97.2 ± 2.7	69.4 ± 10.4	58.3 ± 9.3	55.5 ± 7.5	77.8 ± 7.5	16.6 ± 11.2 b
Bio-82	80.5 ± 6.4	97.2 ± 2.7	88.9 ± 6.3	55.5 ± 8.5	58.3 ± 7.2	75.0 ± 9.3	0.0 ± 0.0 b
Control	94.4 ± 3.7	91.6 ± 4.3	94.4 ± 5.5	75.0 ± 7.2	66.6 ± 5.8	86.1 ± 6.4	50.0 ± 15.1 a
F-values	3.29						
P-value	0.006						

The plant vigor of crops remained relatively consistent across all *Fusarium* isolates, with vigor rates for wheat, barley, lentil, chickpea, pea, and safflower of $74.7 \pm 1.6\%$, $71.4 \pm 2.3\%$, $66.7 \pm 3.3\%$, $66.7 \pm 5.2\%$, $72.5 \pm 3.6\%$, and $70.3 \pm 1.6\%$ respectively (Table 3.6). For comparison, the un-inoculated control had vigor rates of $72.1 \pm 1.4\%$, $68.3 \pm 3.2\%$, $67.9 \pm 2.1\%$, $73.3 \pm 1.3\%$, $74.6 \pm 1.4\%$, and $70.4 \pm 2.2\%$ for wheat, barley, lentil, chickpea, pea, and safflower, respectively. A significant difference in vigor was observed among different crops (ANOVA, $p < 0.05$). No significant difference was observed across isolates for any crop or between the un-inoculated controls (ANOVA, $p > 0.05$). In contrast to the crop results, *Fusarium* isolates significantly affected the vigor of Canada thistle (positive control, ANOVA, $p = 0.03$), with vigor rates of $27.1 \pm 9.0\%$, $47.5 \pm 7.9\%$, and $18.7 \pm 7.4\%$, $38.3 \pm 8.4\%$, $44.2 \pm 9.7\%$, and $26.2 \pm 9.7\%$ for isolates Bio-02, Bio-04, Bio-05 Bio-29, Bio-42, and Bio-82. In contrast, the untreated control had the highest vigor rating of $50.4 (\pm 8.5\%)$. Average plant height was unaffected by *Fusarium* but the positive control Canada thistle had reduced plant height for *Fusarium* treatment ($F = 2.786$, $p = 0.016$).

Table 3.6. Average vigor (%) of various plants to different *Fusarium* isolates and untreated control (ANOVA, $F = 2.48$, $p = 0.03$). Crops had significant differences in overall vigor (Tukey HSD, $\alpha = 0.05$). No significant differences were observed across isolates for any crop or between isolate and the un-inoculated control (Tukey HSD, $\alpha = 0.05$). Significant differences were observed between plant vigor for the inoculated and un-inoculated Canada thistle (ANOVA, $p = 0.03$).

Isolates	Plant Vigor						
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower	Canada thistle
Bio-02	75.4 ± 1.3 a	73.3 ± 1.4 a	71.2 ± 0.9 a	68.7 ± 6.4 a	73.3 ± 2.5 a	73.7 ± 1.5 a	27.1 ± 9.0 bc
Bio-04	74.2 ± 1.3 a	67.5 ± 5.3 a	67.1 ± 2.1 a	66.6 ± 6.2 a	73.7 ± 1.4 a	72.5 ± 1.3 a	47.5 ± 7.9 ab
Bio-05	74.2 ± 1.5 a	71.2 ± 1.5 a	68.7 ± 1.6 a	71.2 ± 1.7 a	75.8 ± 1.2 a	72.9 ± 1.1 a	18.7 ± 7.4 c
Bio-29	75.4 ± 1.4 a	72.5 ± 1.7 a	60.8 ± 6.1 a	67.5 ± 6.3 a	66.6 ± 6.2 a	71.6 ± 1.1 a	38.3 ± 8.4 abc
Bio-42	71.6 ± 1.6 a	70.4 ± 1.9 a	62.5 ± 6.1 a	72.5 ± 1.6 a	68.7 ± 6.4 a	72.1 ± 1.3 a	44.2 ± 9.7 ab
Bio-82	73.7 ± 1.4 a	72.5 ± 1.9 a	62.5 ± 3.5 a	63.3 ± 7.9 a	71.6 ± 2.5 a	65.8 ± 6.1 a	26.2 ± 9.7 bc
UTC	72.1 ± 1.4 a	68.3 ± 2.5 a	67.9 ± 2.1 a	73.3 ± 1.3 a	74.6 ± 1.4 a	70.4 ± 2.2 a	50.4 ± 8.5 a
F-values	2.48						
P-value	0.03						

Field Crop Safety Trial

In both the locations Lutz farm and Fort Ellis, the variable responses like crop emergence, crop vigor and height and yield showed no significant differences between *Fusarium* treated plants and the untreated control for any of the crop species in 2023 and 2024.

Crop Emergence

For Lutz Farm in the year 2023, the average emergence count for wheat, barley, lentil, chickpea, pea, and safflower were 123.4 ± 5.3 , 113.3 ± 6.4 , $62.5.4 \pm 4.3$, 55.4 ± 1.9 , 58.9 ± 2.7 and 125.9 ± 12.5 respectively across the different *Fusarium* isolates. For comparison untreated control (UTC) had emergence rates of 121.6 ± 4.5 , 109.3 ± 5.7 , 57.2 ± 7.6 , 6.6 ± 3.2 , and 127.0 ± 9.4 and 127.00 ± 9.4 for wheat, barley, lentil, chickpea, pea, and safflower, respectively. No significant difference was observed across isolates and crops (ANOVA, $p = 0.95$). In the year 2024, the average crop emergence for wheat, barley, lentil, pea, and safflower was 67.3 ± 6.3 , 64.5 ± 6.7 , 67.1 ± 4.7 , 44.6 ± 4.2 , and 43.8 ± 8.8 , across the different *Fusarium* isolates, respectively. In comparison, the untreated control (UTC) had emergence rates of 62.6 ± 5.6 , 61.0 ± 4.5 , 72.0 ± 6.7 , 44.4 ± 4.0 , and 43.7 ± 2.2 for wheat, barley, lentil, pea, and safflower, respectively. Though a significant difference in emergence counts was observed across treatment, none of the isolate treatments were significantly different from the untreated control (ANOVA, $p = 0.002$; Tukey HSD, $\alpha = 0.05$).

For Fort Ellis Farm in the year 2023, the average crop emergence counts across the different *Fusarium* isolates for wheat, barley, lentil, chickpea, pea, and safflower were 100.0 ± 5.5 , 91.3 ± 7.4 , 80.0 ± 5.4 , 17.2 ± 2.4 , 52.9 ± 1.5 , and 121.0 ± 9.7 , respectively. In comparison, the untreated control (UTC) had emergence rates of 86.1 ± 3.3 , 89.2 ± 6.9 , 84.0 ± 5.9 , 19.4 ± 2.2 , 53.6 ± 1.0 , and 114.4 ± 3.8 for wheat, barley, lentil, chickpea, pea, and safflower, respectively. Though a significant difference in emergence counts was observed across treatment, none of them were significantly different from the untreated control (ANOVA, $p = 0.006$; Tukey HSD, $\alpha = 0.05$). In the year 2024, the average crop emergence counts across the different *Fusarium*

isolates for wheat, barley, lentil, chickpea, pea, and safflower were 71.0 ± 3.9 , 71.2 ± 6.1 , 69.4 ± 4.9 , 16.6 ± 6.0 , 47.8 ± 3.7 , and 71.8 ± 9.0 , respectively. In comparison, the untreated control (UTC) had emergence rates of 78.0 ± 4.0 , 86.2 ± 5.4 , 65.5 ± 1.6 , 11.3 ± 0.6 , 49.4 ± 5.7 , and 59.2 ± 5.6 for wheat, barley, lentil, chickpea, pea, and safflower, respectively. No significant difference was observed across isolate treatments and crops (ANOVA, $p = 0.441$).

Crop Vigor

For Lutz Farm in the year 2023, the average crop vigor across different *Fusarium* isolates for wheat, barley, lentil, chickpea, pea, and safflower were 80.0 ± 0.0 , 78.9 ± 1.1 , 79.1 ± 1.7 , 68.7 ± 2.0 , 80.7 ± 0.8 , and 68.0 ± 1.6 , respectively. In comparison, the untreated control (UTC) had vigor rates of 80.0 ± 0.0 , 78.7 ± 1.2 , 76.2 ± 2.4 , 71.2 ± 1.2 , 80.0 ± 0.0 , and 68.7 ± 1.2 for wheat, barley, lentil, chickpea, pea, and safflower, respectively. No significant difference in crop vigor was observed across isolates and crops (ANOVA, $p = 0.36$). In the year 2024, the average crop vigor across the different *Fusarium* isolates for wheat, barley, lentil, pea, and safflower were 77.0 ± 2.0 , 72.5 ± 2.4 , 78.1 ± 1.6 , 76.8 ± 1.4 , and 75.5 ± 2.3 , respectively. In comparison, the untreated control (UTC) had vigor rates of 80.0 ± 0.0 , 72.5 ± 3.2 , 77.5 ± 2.5 , 75.0 ± 1.6 , and 75.0 ± 2.0 for wheat, barley, lentil, pea, and safflower, respectively. No significant difference in crop vigor was observed across isolate treatments and crops (ANOVA, $p = 0.312$).

For Fort Ellis Farm in the year 2023, the average crop vigor across the different *Fusarium* isolates for wheat, barley, lentil, chickpea, pea, and safflower were 82.7 ± 1.6 , 80.5 ± 0.2 , 75.3 ± 1.3 , 82.1 ± 1.3 , 80.5 ± 0.7 , and 81.4 ± 1.4 , respectively. In comparison, the untreated control (UTC) had vigor rates of 82.5 ± 1.4 , 80.0 ± 0.0 , 77.5 ± 1.4 , 82.5 ± 1.4 , 80.0 ± 0.0 , and 82.5 ± 1.4 for wheat, barley, lentil, chickpea, pea, and safflower, respectively. No significant difference in

crop vigor was observed across isolates and crops (ANOVA, $p = 0.87$). In the year 2024, the average crop vigor across the different *Fusarium* isolates for wheat, barley, lentil, chickpea, pea, and safflower were 76.5 ± 2.1 , 72.5 ± 3.1 , 77.6 ± 1.7 , 75.4 ± 1.0 , 77.0 ± 1.3 , and 74.0 ± 1.7 , respectively. In comparison, the untreated control (UTC) had vigor rates of 77.5 ± 1.4 , 71.2 ± 3.7 , 77.5 ± 1.4 , 75.0 ± 0.0 , 78.7 ± 1.2 , and 72.5 ± 1.4 for wheat, barley, lentil, chickpea, pea, and safflower, respectively. No significant difference in crop vigor was observed across isolate treatments and crops (ANOVA, $p = 0.987$).

Crop Height

For Lutz Farm in the year 2023, the average crop height across the different *Fusarium* isolates for wheat, barley, lentil, chickpea, pea, and safflower were 52.8 ± 1.8 cm, 81.9 ± 2.8 cm, 32.4 ± 1.1 cm, 28.1 ± 4.1 cm, 63.4 ± 2.8 cm, and 54.2 ± 4.4 cm, respectively. In comparison, the untreated control (UTC) had heights of 49.7 ± 1.8 cm, 84.0 ± 2.0 cm, 31.7 ± 0.5 cm, 26.9 ± 3.6 cm, 64.3 ± 2.8 cm, and 56.1 ± 2.4 cm for wheat, barley, lentil, chickpea, pea, and safflower, respectively. No significant difference in crop height was observed across isolates and crops (ANOVA, $p = 0.346$). In the year 2024, the average crop heights across the different *Fusarium* isolates for wheat, barley, lentil, pea, and safflower were 143.7 ± 6.1 cm, 148.9 ± 6.2 cm, 73.7 ± 3.2 cm, 103.8 ± 5.5 cm, and 106.7 ± 5.9 cm, respectively. In comparison, the untreated control (UTC) had heights of 150.4 ± 6.7 cm, 158.1 ± 6.3 cm, 74.1 ± 1.3 cm, 97.2 ± 4.6 cm, and 107.3 ± 6.5 cm for wheat, barley, lentil, pea, and safflower, respectively. No significant difference in crop height was observed across isolate treatments and crops (ANOVA, $p = 0.09$).

For Fort Ellis Farm in the year 2023, the average crop heights across the different *Fusarium* isolates for wheat, barley, lentil, chickpea, pea, and safflower were 63.5 ± 1.9 cm, 89.0

± 2.8 cm, 32.6 ± 1.6 cm, 53.7 ± 1.5 cm, 69.0 ± 3.6 cm, and 61.2 ± 2.5 cm, respectively. In comparison, the untreated control (UTC) had heights of 61.2 ± 2.1 cm, 89.3 ± 1.3 cm, 34.3 ± 0.7 cm, 54.6 ± 1.3 cm, 70.5 ± 1.9 cm, and 62.0 ± 3.6 cm for wheat, barley, lentil, chickpea, pea, and safflower, respectively. No significant difference in crop height was observed across isolates and crops (ANOVA, $p = 0.72$). In the year 2024, the average crop height across the different isolates of *Fusarium* for wheat, barley, lentil, chickpea, pea, and safflower were 149.6 ± 9.7 cm, 170.7 ± 9.7 cm, 80.3 ± 3.1 cm, 104.0 ± 9.9 cm, 123.2 ± 7.1 cm, and 93.3 ± 6.8 cm, respectively. In comparison, the untreated control (UTC) had heights of 153.0 ± 5.4 cm, 180.1 ± 15.2 cm, 82.3 ± 3.3 cm, 99.1 ± 6.7 cm, 122.8 ± 4.0 cm, and 93.1 ± 10.6 cm for wheat, barley, lentil, chickpea, pea, and safflower, respectively. No significant difference in crop height was observed across isolate treatments and crops (ANOVA, $p = 0.59$).

Yield

In 2023, animal damage and late harvesting due to poor ripening of safflower led to shattering of legumes (pea, lentil, and chickpea). This prevented collection of yield data for legumes. The average crop yields across the different *Fusarium* isolates for wheat, barley, and safflower were 1263.2 ± 195.2 g, 1719.3 ± 283.7 g, and 806.9 ± 127.3 g, respectively. In comparison, the untreated control (UTC) had yields of 1302.4 ± 243.8 g, 1674.6 ± 135.4 g, and 782.9 ± 55.9 g for wheat, barley, and safflower, respectively. No significant difference in crop yield was observed across isolates and crops (ANOVA, $p = 0.59$). In the year 2024, the average crop yields across the different *Fusarium* isolates for wheat, barley, lentil, pea, and safflower were 900.2 ± 173.7 g, 899.9 ± 179.5 g, 529.3 ± 74.4 g, 447.2 ± 66.9 g, and 127.7 ± 13.6 g, respectively. In comparison, the untreated control (UTC) had yields of 1095.10 ± 326.8 g, $913.7 \pm$

210.8 g, 552.7 ± 82.5 g, 550.4 ± 64.5 g, and 116.2 ± 10.1 g for wheat, barley, lentil, pea, and safflower, respectively. No significant difference in crop yield was observed across isolate treatments and crops (ANOVA, $p = 0.88$). At Lutz Farm, chickpea emergence was severely impacted by high *Pythium* levels in the soil, resulting in no yield data.

For Fort Ellis Farm in the year 2023, the average crop yields across the different *Fusarium* isolates for wheat, barley, pea, and safflower were 1832.6 ± 124.9 g, 1127.1 ± 179.7 g, 119.4 ± 14.8 g, and 1150.5 ± 104.9 g, respectively. In comparison, the untreated control (UTC) had yields of 1500.5 ± 202.97 g, 1217.3 ± 138.0 g, 124.5 ± 14.3 g, and 1276.2 ± 88.6 g for wheat, barley, pea, and safflower, respectively. No significant difference in crop yield was observed across isolates and crops (ANOVA, $p = 0.24$). For Fort Ellis farm in the year 2024, the average crop yields across the different *Fusarium* isolates for wheat, barley, lentil, chickpea, pea, and safflower were 1089.3 ± 202.1 g, 346.1 ± 99.5 g, 510.2 ± 82.5 g, 362.2 ± 120.3 g, 401.7 ± 80.6 g, and 183.1 ± 43.5 g, respectively. In comparison, the untreated control (UTC) had yields of 1331.2 ± 100.6 g, 378.8 ± 100.2 g, 568.6 ± 124.5 g, 175.9 ± 24.8 g, 452.6 ± 106.7 g, and 129.3 ± 7.2 g for wheat, barley, lentil, chickpea, pea, and safflower, respectively. No significant difference in crop yield was observed across isolate treatments and crops (ANOVA, $p = 0.97$).

Discussion

The exploration of biological control agents for managing invasive weeds like Canada thistle (*Cirsium arvense*) has garnered significant attention due to the limitations and environmental concerns associated with chemical herbicides. In this context, our research evaluated the efficacy and crop safety of *Fusarium oxysporum* and *Fusarium redolens* isolates as potential biocontrol agents against Canada thistle.

Successes in the greenhouse did not translate to consistent successes in the field. Our greenhouse experiments demonstrated that both *F. oxysporum* and *F. redolens* isolates effectively suppressed Canada thistle growth. These findings were corroborated by field trials conducted in 2023 in Highwood, where *Fusarium oxysporum* isolates exhibited significant control over Canada thistle populations. While not significant, similar control patterns were observed at the Big Timber location. The success of *F. oxysporum* aligns with previous studies highlighting its potential as a biocontrol agent for various weeds such as *Opuntia*, *Cannabis*, *Striga* (Fullaway, 1954; Wilson, 1969; Tiourebaev, 2001; Punja, 2021; Venne et al., 2009; Lüth et al., 2016). Unfortunately, in 2024, no effect was observed from any of the inoculations. In 2024, the average precipitation recorded was 1.80 inches, while in 2023, it reached 2.50 inches at Highwood location, representing an increase of 38.89% compared to 2024 (Great Falls, MT Weather History | Weather Underground). This difference in rainfall may have influenced the survival and spread of *Fusaria* inoculum during the year 2024. Greenhouse conditions provide stable and optimal environments, such as consistent temperature, humidity, and light, which favor both the biocontrol agents and the target weed (Anttila et al., 2016). In contrast, field conditions are inherently variable, with fluctuating environmental factors such as temperature, soil moisture, and microbial competition, which can adversely impact the survival, dispersal, and efficacy of biocontrol agents (De Curtis et al., 2012). The variable environmental conditions in Montana, including its cooler and drier climate, also likely limited the efficacy of *Fusarium* in field trials. *Fusarium* thrives in warm, moist conditions (Okungbowa & Shittu, 2012), but lower humidity and colder temperatures in Montana may have reduced its ability to establish and maintain infection levels sufficient to suppress Canada thistle (Ortega et al., 2019). Additionally,

competition from native soil microorganisms and antagonistic interactions could further weaken *Fusarium's* efficacy (Köhl et al., 2019). On top of that, we employed a specific genotype of Canada thistle in our greenhouse experiments, while the thistle present in the field may be a different genotype, potentially exhibiting varied responses to the pathogen.

While both fungal isolates were effective against Canada thistle, their crop safety profiles differed. In initial greenhouse crop safety trials, *F. redolens* adversely affected wheat, barley, and legumes, indicating a lack of host specificity. This observation is consistent with literature reports where *F. redolens* has been associated with diseases of lentil, chickpea, pea wheat, and soybean (Bienapfl, 2010; Jiménez-Fernández, 2011; Esmaceli Taheri, 2011). While follow up greenhouse crop safety trials failed to reproduce the *F. redolens* effects on these crops, overall efficacy of inoculums in these trials was lower as observed in the Canada thistle controls. In contrast to *F. redolens*, *F. oxysporum* isolates did not exhibit detrimental effects on these crops, suggesting a higher degree of host specificity and safety. This specificity is crucial for biocontrol agents to minimize unintended impacts on non-target plant species.

To enhance the field efficacy of *Fusarium*, strategies such as combining biocontrol agents with cultural or chemical methods, genetic improvements to enhance environmental resilience, or improved formulations to withstand field conditions may be explored (St Leger & Screen, 2001; Gao & Su, 2024). Future research should prioritize understanding and addressing the ecological and environmental factors limiting biocontrol agents' effectiveness in field conditions, with a focus on improving persistence, delivery methods, and integration into broader pest management practices. These findings emphasize the importance of bridging the gap between

greenhouse successes and field applications, ultimately paving the way for sustainable weed management solutions.

In conclusion, greenhouse trials suggest both *F. redolens* and *F. oxysporum* represent significant pathogens on Canada thistle. Unfortunately, *F. redolens* isolates displayed significant pathogenicity on peas, lentils, chickpeas and barley. In contrast, *F. oxysporum* isolates displayed strong thistle specificity, which makes for an ideal biological control candidate. Initial field efficacy trials with *F. oxysporum* isolates displayed significant suppression of Canada thistle but subsequent trials failed to suppress. Mechanisms behind success and failure for *F. oxysporum* are unclear at this time but given the initial successes, warrants additional investigations.

References Cited

- Anttila, J., Laakso, J., Kaitala, V., & Ruokolainen, L. (2016). Environmental variation enables invasions of environmental opportunist pathogens. *Oikos*, 125(8), 1144-1152.
- Baker, B. P., & Mohler, C. L. (2015). Weed management by upstate New York organic farmers: Strategies, techniques and research priorities. *Renewable agriculture and food systems*, 30(5), 418-427.
- Bienapfl, J. C., Malvick, D. K., & Percich, J. A. (2010). First report of *Fusarium redolens* causing root rot of soybean in Minnesota. *Plant Disease*, 94(8), 1069-1069.
- Charudattan, R., & Dinoor, A. (2000). Biological control of weeds using plant pathogens: accomplishments and limitations. *Crop Protection*, 19(8-10), 691-695.
- Dagno, K., Lahlali, R., Diourté, M., & Jijakli, H. (2012). Present status of the development of mycoherbicides against water hyacinth: successes and challenges. A review. *Biotechnologie, Agronomie, Société et Environnement*, 16(3).
- De Curtis, F., De Felice, D. V., Ianiri, G., De Cicco, V., & Castoria, R. (2012). Environmental factors affect the activity of biocontrol agents against ochratoxigenic *Aspergillus carbonarius* on wine grape. *International journal of food microbiology*, 159(1), 17-24.
- Donald, W.W. (1994). Geostatistics for mapping weeds, with a Canada thistle (*Cirsium arvense*) patch as a case study. *Weed Science*, 42: 648-657.
- Enloe, S. F., Lym, R. G., Wilson, R., Westra, P., Nissen, S., Beck, G., Halstvedt, M. (2007). Canada thistle (*Cirsium arvense*) control with aminopyralid in range, pasture, and noncrop Areas. *Weed Technology*, 21(4), 890–894. doi:10.1614/WT-07-004.1
- Esmaeili Taheri, A., Hamel, C., Gan, Y., & Vujanovic, V. (2011). First report of *Fusarium redolens* from Saskatchewan and its comparative pathogenicity. *Canadian Journal of Plant Pathology*, 33(4), 559-564.
- Fullaway, D.T. (1954). Biological control of cactus in Hawaii. *Journal of Economic Entomology* 47:696-700
- Gao, W. T., & Su, W. H. (2024). Weed Management Methods for Herbaceous Field Crops: A Review. *Agronomy*, 14(3), 486.
- Ghorbani, R., Leifert, C., & Seel, W. (2005). Biological control of weeds with antagonistic plant pathogens. *Advances in Agronomy*, 86, 191-225.

- Great Falls, MT Weather History | Weather Underground. Wunderground.com, 2024, www.wunderground.com/history/monthly/us/mt/highwood/KGTF/date/2024-9. Accessed 22 Dec. 2024.
- Jiménez-Fernández, D., Navas-Cortés, J. A., Montes-Borrego, M., Jiménez-Díaz, R. M., & Landa, B. B. (2011). Molecular and pathogenic characterization of *Fusarium redolens*, a new causal agent of *Fusarium* yellows in chickpea. *Plant Disease*, 95(7), 860-870.
- Kookana, R. S., Baskaran, S. N. R. S. N. R., & Naidu, R. (1998). Pesticide fate and behaviour in Australian soils in relation to contamination and management of soil and water: a review. *Soil Research*, 36(5), 715-764.
- Köhl, J., Kolnaar, R., & Ravensberg, W. J. (2019). Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. *Frontiers in plant science*, 10, 845.
- Lüth, P., Nzioki, H. S., Sands Baker, C., & Sands, D. C. (2024). A microbial bioherbicide for *Striga hermonthica* control: production, development, and effectiveness of a seed coating agent. *Pest Management Science*, 80(1), 149-155.
- Miller, B. R., & Lym, R. G. (2017). Using the rosette technique for Canada thistle (*Cirsium arvense*) control in row crops. *Weed Technology*, 12(4), 699–706. doi:10.1017/S0890037X00044572
- Okungbowa, F. I., & Shittu, H. O. (2012). *Fusarium* wilts: An overview. *Environ. Res. J*, 6(2), 83-102.
- Ortega, L. M., Romero, L., Moure, C., Garmendia, G., Albuquerque, D. R., Pinto, V. F., Vero, S., & Alconada, T. M. (2019). Effect of moisture on wheat grains lipid patterns and infection with *Fusarium graminearum*. *International journal of food microbiology*, 306, 108264.
- Ringselle, B., Brandsæter, L. O., Mangerud, K., & Bergkvist, G. (2023). Vertical rhizome disking to reduce *Elymus repens* (quackgrass) abundance in grass-clover leys. *Crop Protection*, 172, 106301.
- St Leger, R., & Screen, S. (2001). Prospects for strain improvement of fungal pathogens of insects and weeds.
- Stachon, W. J., & Zimdahl, R. L. (1980). Allelopathic Activity of Canada Thistle (*Cirsium arvense*) in Colorado. *Weed Science*, 28(1), 83–86. doi:10.1017/S004317450002782X.
- Van Dam, P., de Sain, M., Ter Horst, A., van der Gragt, M., & Rep, M. (2018). Use of comparative genomics-based markers for discrimination of host specificity in *Fusarium oxysporum*. *Applied and environmental microbiology*, 84(1), e01868-17.

- Venne, J., Beed, F., Avocanh, A., & Watson, A. (2009). Integrating *Fusarium oxysporum f. sp. strigae* into cereal cropping systems in Africa. *Pest Management Science: Formerly Pesticide Science*, 65(5), 572-580.
- Wilson, C.L. (1969). Use of plant pathogens in weed control. *Annual Review of Phytopathology* 7:411-434.

CHAPTER FOUR

SUMMARY

The management of invasive weeds like Canada thistle (*Cirsium arvense*) presents a significant challenge due to the limitations of chemical herbicides, including environmental concerns and resistance development. Our research focused on identifying and evaluating potential biocontrol agents, specifically *Fusarium oxysporum* and *Fusarium redolens*, for their pathogenicity, efficacy, and crop safety.

A limited range of fungal species were isolated from diseased Canada thistle, suggesting a relatively simple pathological interaction dominated by a few pathogens. Among the fungi identified, unknown *Alternaria* species and *Fusarium oxysporum* and *Fusarium redolens* emerged as likely pathogenic agents, with other fungi identified primarily as saprophytes. Notably, *F. oxysporum* and *F. redolens* represented a significant portion of the isolates, and their aggressive pathogenicity was confirmed through greenhouse inoculation trials (Kruskal-Wallis $\chi^2 = 20.92$, $p < 0.001$) and the fulfillment of Koch's postulates.

Greenhouse efficacy trials demonstrated that both *F. oxysporum* and *F. redolens* effectively suppressed Canada thistle growth ($\chi^2 = 34.85$, $p < 0.001$). *Fusarium oxysporum* isolate Bio-05 and *F. redolens* isolate Bio-04 had highest suppression of Canada thistle in the greenhouse trials. Field trials conducted in Montana further supported the efficacy of *F. oxysporum*, although its performance was influenced by environmental variability, such as the cooler and drier climate. In contrast, *F. redolens* exhibited adverse effects on emergence, vigor and biomass on non-target crops like wheat, lentil, and chickpea, aligning with its known pathogenicity in pulse crops and raising concerns about its lack of host specificity.

These observations highlight *F. oxysporum*'s potential as a safer and more selective biocontrol agent compared to *F. redolens*. Its lack of adverse effects on key crops suggests it could be integrated into weed management systems with minimal risk to non-target species. However, challenges remain in translating greenhouse successes to field conditions. Environmental factors such as soil properties, microbial competition, and climate variability significantly impact the survival, dispersal, and efficacy of these fungi in natural settings. Additionally, the relationship between these pathogens and various genotypes of Canada thistle requires further investigation.

In conclusion, while *F. oxysporum* and *F. redolens* both exhibit potential as biocontrol agents, the former stands out due to its greater crop safety and specificity. Continued research to address field efficacy and environmental adaptability could establish *F. oxysporum* as a cornerstone of sustainable weed management strategies, reducing reliance on chemical herbicides and promoting agricultural sustainability.

REFERENCES CITED

- Al-Khafaji, R. T., Gunnink Troth, E. E., Lambert, K. N., Johnston, J. A., & Dyer, A. T. (2019). Pathotypes detected among populations of *Pratylenchus neglectus* collected from Montana. *Plant disease*, 103(12), 3259-3264.
- All, G.M.B. (1975). The allelopathic activity of Californian thistle (*Cirsium arvense* (L.) Scop.) in Tasmania. *Weed Research*, 15, 77-81
- Alley, H. P. (1981). Mechanical, cultural, and chemical control of Canada thistle in small grains and pastures. In *Proc. North Cent. Weed Control Conf*, 36, 176-179.
- Anttila, J., Laakso, J., Kaitala, V., & Ruokolainen, L. (2016). Environmental variation enables invasions of environmental opportunist pathogens. *Oikos*, 125(8), 1144-1152.
- Army, A.C. (1932). Variations in the organic reserves in underground parts of five perennial weeds from late April to November. *Minnesota Agricultural Experiment station's Bulletin* No. 84, 28.
- Bacher, S., Heitzmann, A., Nentwig, W. (1997). Problemunkräuter in ökologischen Ausgleichsflächen im Ackerbau. *Agrarforschung* 4, 65–67.
- Bachthaler, G., Kees, H., & Dinzenhofer, B. (1983). The build-up of resistant lines of arable weeds after continued use of herbicides, especially triazines. Present state of knowledge of causes and practical consequences.
- Beck, K.G., (2018). Canada Thistle -Extension, 3-108
- Bienapfl, J. C., Malvick, D. K., & Percich, J. A. (2010). First report of *Fusarium redolens* causing root rot of soybean in Minnesota. *Plant Disease*, 94(8), 1069-1069.
- Boari, Angela., & Maurizio, Vurro. (2004). Evaluation of *Fusarium* spp. And Other Fungi as Biological Control Agents of Broomrape (*Orobanche Ramosa*). *Biological Control*, 30, 212–219, <https://doi.org/10.1016/j.biocontrol.2003.12.003>. Accessed 1 Nov. 2021.
- Boerboom, C. M., & Wyse, D. L. (1988). Selective application of herbicides for Canada thistle (*Cirsium arvense*) control in birdsfoot trefoil (*Lotus corniculatus*). *Weed Technology*, 2(2), 183-186.
- Bowman, R. A., & Halvorson, A. D. (1997). Crop rotation and tillage effects on phosphorus distribution in the central Great Plains. *Soil Science Society of America Journal*, 61(5), 1418-1422.
- Boyette, C. D., Templeton, G. E., & Oliver, L. R. (1984). Texas Gourd (*Cucurbita texana*) Control with *Fusarium solani* f. sp. *cucurbitae*. *Weed Science*, 32(5), 649–655. doi:10.1017/S0043174500059737.

- Bybd Jr, D. W., Kirkpatrick, T., & Barker, K. (1983). An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of nematology*, 15(1), 142.
- Canada thistle biocontrol | Department of Agriculture. (n.d.). Retrieved December 5, 2024, from <https://ag.colorado.gov/conservation/biocontrol/canada-thistle>
- Carter, TR., and Lym, R.G. (2017). Canada thistle (*Cirsium arvense*) affects herbage production in the Northern Great Plains. *Invasive Plant Science and Management*. 10(4): 332-339. doi:10.1017/inp.2017.34.
- Chad W, Grekul., and Edward W, Bork. (2004). Herbage yield losses in perennial pasture due to Canada thistle (*Cirsium arvense*). *Weed Technology*, 18(3), 784-794. <https://doi.org/10.1614/WT-03-196R>
- Charudattan, R., & Dinoor, A. (2000). Biological control of weeds using plant pathogens: accomplishments and limitations. *Crop Protection*, 19(8-10), 691-695.
- Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y., & Zhou, S. (2016). Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. *Molecular ecology resources*, 16(1), 138-149.
- Cripps, M. G., Gassmann, A., Fowler, S. V., Bourdôt, G. W., McClay, A. S., & Edwards, G. R. (2011). Classical biological control of *Cirsium arvense*: lessons from the past. *Biological Control*, 57(3), 165-174.
- Dagno, K., Lahlali, R., Diourte, M., & Jijakli, H. (2012). Present status of the development of mycoherbicides against water hyacinth: successes and challenges. A review. *Biotechnologie, Agronomie, Société et Environnement*, 16(3).
- Darwent, A. L., Kirkland, K. J., Baig, M. N., & Lefkovitch, L. P. (1994). Preharvest applications of glyphosate for Canada thistle (*Cirsium arvense*) control. *Weed technology*, 8(3), 477-482.
- De Curtis, F., De Felice, D. V., Ianiri, G., De Cicco, V., & Castoria, R. (2012). Environmental factors affect the activity of biocontrol agents against ochratoxigenic *Aspergillus carbonarius* on wine grape. *International journal of food microbiology*, 159(1), 17-24.
- Dewey, L.H. (1901). Canada thistle [*Carduus arvensis* (L.) Robs.]. Washington, DC: USDA Division of Botany, 27. 14.
- Donald, W. W., & Khan, M. (1992). Yield loss assessment for spring wheat (*Triticum aestivum*) infested with Canada thistle (*Cirsium arvense*). *Weed Science*, 40(4), 590-598.
- Donald, W.W. (1994). Geostatistics for mapping weeds with a Canada thistle (*Cirsium arvense*) patch as a case study. *Weed Science*, 42, 648-657.

- Eberly, J.O., Hurd, A., Oli, D., Dyer, A.T., Seipel, T.F., and Carr, P.M. (2024). Compositional profiling of the rhizosphere microbiome of Canada thistle reveals consistent patterns across the United States northern Great Plains. *Sci Rep*, 14, 18016. <https://doi.org/10.1038/s41598-024-69082-3>
- Edwards, GR., Bourdôt. G.W., and Crawley. MJ. (2000). Influence of herbivory, competition and soil fertility on the abundance of *Cirsium arvense* in acid grasslands. *Journal of Applied Ecology*, 37: 321–334. doi: 10.1046/j.1365-2664.2000.00495.x
- Eilenberg, J., Hajek. A., and Lomer. C. (2001). Suggestions for unifying the terminology in biological control. *Biocontrol Sci Technol*, 46:387–400.
- Enloe, S. F., Lym, R. G., Wilson, R., Westra, P., Nissen, S., Beck, G., Halstvedt, M. (2007). Canada thistle (*Cirsium arvense*) control with aminopyralid in range, pasture, and noncrop Areas. *Weed Technology*, 21(4), 890–894. doi:10.1614/WT-07-004.1
- Esmaili Taheri, A., Hamel, C., Gan, Y., & Vujanovic, V. (2011). First report of *Fusarium redolens* from Saskatchewan and its comparative pathogenicity. *Canadian Journal of Plant Pathology*, 33(4), 559-564.
- Ferrer, C., Colom, F., Frases, S., Mulet, E., Abad, J. L., & Alió, J. L. (2001). Detection and identification of fungal pathogens by PCR and by ITS2 and 5.8 S ribosomal DNA typing in ocular infections. *Journal of clinical microbiology*, 39(8), 2873-2879.
- Fogelfors, H., & Lundkvist, A. (2008). Selection in *Cirsium arvense* (L.) Scop. and *Sonchus arvensis* L.: Susceptibility to MCPA on different types of farmland in Sweden. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, 58(1), 82-87
- Frank, J. R., & Tworkoski, T. J. (1994). Response of Canada thistle (*Cirsium arvense*) and leafy spurge (*Euphorbia esula*) clones to chlorsulfuron, clopyralid, and glyphosate. *Weed technology*, 8(3), 565-571.
- Frantzen, J. (1994). The role of clonal growth in the pathosystem *Cirsium arvense* *Puccinia punctiformis*. *Canadian Journal of Botany*, 72: 832–836. doi: 10.1139/b94-107
- French, R.C., and Lightfield. AR. (1990). Induction of systemic aecial infection in Canada thistle (*Cirsium arvense*) by teliospores of *Puccinia punctiformis*. *Phytopathology*, 80: 872–877. doi: 10.1094/Phyto-80-872
- French, R.C., Turner, S.K., Sonnett, P.E., Pfeffer, P., and Piotrowski, E. (1988). Properties of an extract from Canada thistle roots that stimulates germination of dormant teliospores of Canada thistle rust (*Puccinia punctiformis*). *Journal of Agriculture and Food Chemistry* 36: 1043–1047. doi: 10.1021/jf00083a034

- Frey, J. E., & Frey, B. (1995). Molecular identification of six species of scale insects (*Quadraspidiotus sp.*) by RAPD-PCR: assessing the field-specificity of pheromone traps. *Molecular ecology*, 4(6), 777-780.
- Fullaway, D.T. (1954). Biological control of cactus in Hawaii. *Journal of Economic Entomology* 47:696-700
- Gao, W. T., & Su, W. H. (2024). Weed Management Methods for Herbaceous Field Crops: A Review. *Agronomy*, 14(3), 486.
- Ghorbani, R., Leifert, C., & Seel, W. (2005). Biological control of weeds with antagonistic plant pathogens. *Advances in Agronomy*, 86, 191-225.
- Haderlie, L. C., McAllister, R. S., Hoefer, R. H., & Leino, P. W. (1992). Canada thistle control. In *Noxious Range Weeds*, 260-263
- Halsey, W. S. (1992). Responses of Canada thistle to defoliation and to damage from a stem-mining weevil in Utah.
- Halsted, B. D. (1894). Weeds and their most common fungi. *New Jersey Exp. Stn. Rep.*, 1002.
- Heap, I. (2014). Global perspective of herbicide-resistant weeds. *Pest Manag. Sci.* 70, 1306–1315. doi:10.1002/ps.3696
- Heinisch, O. (1931). Die Ackerkratzdistel. *Cirsium arvense* (L.) Scop. In Roemer, Th. (ed.) *Archiv für Pflanzenbau*. Verlag von Julius Springer, Berlin, 348–420.
- Hodgson, J. M. (1970). The response of Canada thistle ecotypes to 2, 4-D, amitrole, and intensive cultivation. *Weed Science*, 18(2), 253-255.
- Hodgson, J.M. (1968). The nature, ecology, and control of Canada thistle. *Technical Bulletin of the United States Department of Agriculture*, No. 1386. United States Department of Agriculture, Washington, DC.
- Holterman, M. H., Oggenfuss, M., Frey, J. E., & Kiewnick, S. (2012). Evaluation of high-resolution melting curve analysis as a new tool for root-knot nematode diagnostics. *Journal of Phytopathology*, 160(2), 59-66.
- Hurrell, G.A., and Bourdot, G.W. (1996). *Sclerotinia sclerotiorum* and mowing independently reduce Californian thistle in a sheep pasture. *New Zealand Plant Protection Society*, Rotorua, NZ. ISSG (2011) Invasive species specialist group.
- Jacobs, J., Sciegienka, J., & Menalled, F. D. (2006). *Ecology and Management of Canada Thistle (Cirsium arvense (L.) Scop.)*. US Department of Agriculture, Natural Resources Conservation Service.

- Jalali, B.L., and Chand, H. (1992). Chickpea wilt. In: Plant Diseases of International Importance. Vol. 1. Diseases of Cereals and Pulses. U. S. Singh, A.N., Mukhopadhyay, J. Kumar., and H. S. Chaube, eds. Prentice Hall, Englewood Cliffs, NY, USA. p. 429-444
- Jiménez-Fernández, D., Navas-Cortés, J. A., Montes-Borrego, M., Jiménez-Díaz, R. M., & Landa, B. B. (2011). Molecular and pathogenic characterization of *Fusarium redolens*, a new causal agent of *Fusarium* yellows in chickpea. *Plant Disease*, 95(7), 860-870.
- K.G., Beck. (2018). Canada Thistle -Extension, 3-108
- Kluth, S., Kruess, A., and Tschardt, T. (2005). Effects of two pathogens on the performance of *Cirsium arvense* in a successional fallow. *Weed Research* 45: 261–269. doi:10.1111/j.1365- 3180.2005.00463.x
- Köhl, J., Kolnaar, R., & Ravensberg, W. J. (2019). Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. *Frontiers in plant science*, 10, 845.
- Kohlschmid, E., Sauerborn, J., & Müller-Stöver, D. (2009). Impact of *Fusarium oxysporum* on the holoparasitic weed *Phelipanche ramosa*: biocontrol efficacy under field-grown conditions. *Weed research*, 49, 56-65.
- Kurtz, Cassandra M. (2013). An Assessment of Invasive Plant Species Monitored by the Northern Research Station Forest Inventory and Analysis Program, 2005 through 2010. USDA, 43136. <https://doi.org/10.2737/nrs-gtr-109>
- Larson, G. E., Wittig, T. A., Higgins, K. F., Turnipseed, B., & Gardner, D. M. (2005). Influence of biocontrol insects on Canada thistle: seed production, germinability, and viability.
- Liebman, M., & Davis, A. S. (2009). Managing weeds in organic farming systems: an ecological approach. *Organic farming: the ecological system*, 54, 173-195.
- Logrieco, A., Moretti, A., & Solfrizzo, M. (2009). *Alternaria* toxins and plant diseases: an overview of origin, occurrence and risks. *World Mycotoxin Journal*, 2(2), 129-140.
- Lüth, P., Nzioki, H. S., Sands Baker, C., & Sands, D. C. (2024). A microbial bioherbicide for *Striga hermonthica* control: production, development, and effectiveness of a seed coating agent. *Pest Management Science*, 80(1), 149-155.
- Ma, L.-J., Geiser, D. M., Proctor, R. H., Rooney, A. P., O'Donnell, K., Trail, F., & Kistler, H. C. (2013). *Fusarium* pathogenomics. *Annual Review of Microbiology*, 67, 399-416.
- Mangold, J. M., Fuller, K. B., Davis, S. C., & Rinella, M. J. (2018). The Economic Cost of Noxious Weeds on Montana Grazing Lands. *Invasive Plant Science and Management*, 11(2), 96–100. doi:10.1017/inp.2018.10

- Marley, P. S., Kroschel, J., & Elzien, A. (2005). Host specificity of *Fusarium oxysporum* Schlecht (isolate PSM 197), a potential mycoherbicide for controlling *Striga* spp. in West Africa. *Weed research*, 45(6), 407-412.
- McAllister, R.S., and Haderlie, L.C. (1985). Seasonal variations in Canada thistle (*Cirsium arvense*) root bud growth and root carbohydrate reserves. *Weed Science* 33:44–49.
- McLennan, B. R., et al. (1991). *Cirsium Arvense* (L.) Scop. Competition with Winter Wheat (*Triticum Aestivum* L.). *Weed Research*, 31(6), 409–415.
<https://doi.org/10.1111/j.1365-3180.1991.tb01781.x>
- Michielse, C. B., & Rep, M. (2009). Pathogen profile update: *Fusarium oxysporum*. *Molecular Plant Pathology*, 10(3), 311-324.
- Miller, B. R., & Lym, R. G. (2017). Using the rosette technique for Canada thistle (*Cirsium arvense*) control in row crops. *Weed Technology*, 12(4), 699–706.
doi:10.1017/S0890037X00044572
- Moore, R. J. (1975). The biology of Canadian weeds: *Cirsium arvense* (L.) Scop. *Can. J. Plant Sci.* 55, 13, 1033–1048. doi:10.4141/cjps75-163
- Moore, R.J., & Frankton, C. (1965). Cytotaxonomy of *Cirsium hookerianum* and related species. *Canadian Journal of Botany*, 43, 597–613.
- Moyer, J. R., Schaalje, G. B., & Bergen, P. (1991). Alfalfa (*Medicago sativa*) seed yield loss due to Canada thistle (*Cirsium arvense*). *Weed Technology*, 5(4), 723-728.
- Müller, E., Jud, P., and Nentwig, W. (2011). Artificial infection of *Cirsium arvense* with the rust pathogen *Puccinia punctiformis* by imitation of natural spore-transfer of the weevil *Ceratapion onopordi*. *Weed Research*, doi: 10.1111/j.1365-3180.2010.00839.x
- Nadeau, L. B., and H, Vanden. (1989). The root system of Canada thistle. *Canadian Journal of Plant Science*, 69(4), 1199–1206, <https://doi.org/10.4141/cjps89-142>
- Nazer, Kakhaki., S. H., et al. (2017). Biocontrol of Broomrape Using *Fusarium Oxysporum* F. Sp. *Orthoceras* in Tomato Crops under Field Conditions. *Biocontrol Science and Technology*, 27(12), 1435–1444, <https://doi.org/10.1080/09583157.2017.1409338>
- Nelson, P. E., Desjardins, A. E., & Plattner, R. D. (1993). Fumonisin, mycotoxins produced by *Fusarium* species: biology, chemistry, and significance.
- Okungbowa, F. I., & Shittu, H. O. (2012). *Fusarium* wilts: An overview. *Environ. Res. J.*, 6(2), 83-102.

- Orloff, N., Mangold, J., Miller, Z., & Menalled, F. (2018). A meta-analysis of field bindweed (*Convolvulus arvensis* L.) and Canada thistle (*Cirsium arvense* L.) management in organic agricultural systems. *Agriculture, Ecosystems & Environment*, 254, 264-272.
- Ortega, L. M., Romero, L., Moure, C., Garmendia, G., Albuquerque, D. R., Pinto, V. F., Vero, S., & Alconada, T. M. (2019). Effect of moisture on wheat grains lipid patterns and infection with *Fusarium graminearum*. *International journal of food microbiology*, 306, 108264.
- O'Sullivan, P.A., Kossatz, V.C., Weiss, G.M., and Dew, D.A. (1982). An approach to estimating yield loss of barley due to Canada thistle. *Can J Plant Sci*, 62:125-131.
<https://doi.org/10.4141/cjps82-105>
- O'Sullivan, P.A., Weiss, G.M., and Kossatz, V.C. (1985). Indices of competition for estimating rapeseed yield loss due to Canada thistle. *Can J Plant Sci* 65:145–149.
<https://doi.org/10.4141/cjps85-020>
- Özdemir, F. G. G., Yaşar, B., Arıcı, Ş. E., & Elekcioglu, İ. H. (2022). Interaction effect of root lesion nematodes and *Fusarium culmorum* Sacc. on disease complex in some wheat cultivars. *Yuzuncu Yıl University Journal of Agricultural Sciences*, 32(1), 152-163.
- Peschken, D. P., & Wilkinson, A. T. S. (1981). Biocontrol of *C. arvense* (*Cirsium arvense*): releases and effectiveness of *Ceutorhynchus litura* (Coleoptera: Curculionidae) in Canada. *Canadian Entomologist*, 113(9), 777–785.
- Pilipavičius, V., and Romaneckas, K. (2014). Allelopathic activity of creeping thistle water extracts on germination and early growth of winter wheat. *Bulgarian Journal of Agricultural Science* 20(3), 607–612 doi: 20.500.12259/87144
- Pleban, S., & Strobel, G. A. (1998). Rapid evaluation of *Fusarium* spp. as a potential biocontrol agent for weeds. *weed Science*, 46(6), 703-706.
- Punja, Z. K., & Rodriguez, G. (2018). *Fusarium* and *Pythium* species infecting roots of hydroponically grown marijuana (*Cannabis sativa* L.) plants. *Canadian Journal of Plant Pathology*, 40(4), 498-513.
- Ringselle, B., Brandsæter, L. O., Mangerud, K., & Bergkvist, G. (2023). Vertical rhizome disking to reduce *Elymus repens* (quackgrass) abundance in grass-clover leys. *Crop Protection*, 172, 106301.
- Royer, F., and R. Dickinson. (1999). *Weeds of the Northern U.S. and Canada*. The University of Alberta press. 434
- Shishido, M., Miwa, C., Usami, T., Amemiya, Y., & Johnson, K. B. (2005). Biological control efficiency of *Fusarium* wilt of tomato by nonpathogenic *Fusarium oxysporum* Fo-B2 in different environments. *Phytopathology*, 95(9), 1072-1080.

- Solymosi, P., & Nagy, P. (1998). ALS-resistance in *Cirsium arvense* (L.) scop.
- St Leger, R., & Screen, S. (2001). Prospects for strain improvement of fungal pathogens of insects and weeds.
- Stachon, W. J., & Zimdahl, R. L. (1980). Allelopathic Activity of Canada Thistle (*Cirsium arvense*) in Colorado. *Weed Science*, 28(1), 83–86. doi:10.1017/S004317450002782X
- Stevens, A. (1846). Extirpation of Canada thistles. *Extirpation of Canada thistles*. 406–428.
- Taheri, A. (1996). Interaction between root lesion nematode, *Pratylenchus neglectus*, and root-rotting fungi of wheat (Doctoral dissertation).
- Tan, W. Z., Li, Q. J., & Qing, L. (2002). Biological control of alligatorweed (*Alternanthera philoxeroides*) with a *Fusarium* sp. *Bio Control*, 47, 463-479.
- Thomma, B. P. (2003). *Alternaria spp.*: from general saprophyte to specific parasite. *Molecular plant pathology*, 4(4), 225-236.
- Thompson, J. P., & Clewett, T. G. (2021). Investigating the impact of root-lesion nematodes (*Pratylenchus thornei*) and crown rot (*Fusarium pseudograminearum*) on diverse cereal cultivars in a conservation farming system. *Agronomy*, 11(5), 867.
- Tiley, G.E. (2010). Biological flora of the British Isles: *Cirsium arvense* (L.) Scop. *J Ecol* 98(4):938–983. <https://doi.org/10.1111/j.1365-2745.2010.01678.x>
- Tiourebaev, K. S., Semenchenko, G. V., Dolgovskaya, M., McCarthy, M. K., Anderson, T. W., Carsten, L. D., Pilgeram, A.L., & Sands, D. C. (2001). Biological control of infestations of ditchweed (*Cannabis sativa*) with *Fusarium oxysporum* f. sp. *cannabis* in Kazakhstan. *Biocontrol science and technology*, 11(4), 535-540.
- Van Dam, P., de Sain, M., Ter Horst, A., van der Gragt, M., & Rep, M. (2018). Use of comparative genomics-based markers for discrimination of host specificity in *Fusarium oxysporum*. *Applied and environmental microbiology*, 84(1), e01868-17.
- Van den, Ende G., Frantzen, J., Timmers, T., (1987) Teleutospores as origin of systemic infection of *Cirsium arvense* by *Puccinia punctiformis*. *Netherland Journal of Plant Pathology* 93: 233–239. doi: 10.1007/BF01998251
- Van Leest, AS., and Scheepens, P.C. (1994). Effectiveness of the rust fungus *Puccinia punctiformis* as a possible biological control agent against Canada thistle (*Cirsium arvense*). *Rapport- DLO Instituut voor Agrobiologisch en Bodemvruchtbaarheidsonderzoek* zoek 25: 1–19.

- Venne, J., Beed, F., Avocanh, A., & Watson, A. (2009). Integrating *Fusarium oxysporum* f. *sp. strigae* into cereal cropping systems in Africa. *Pest Management Science: Formerly Pesticide Science*, 65(5), 572-580.
- Verbeek, J.D., and Kotanen, P.M. (2019). Soil-mediated impacts of an invasive thistle inhibit the recruitment of certain native plants. *Oecologia*, 190, 619–628.
<https://doi.org/10.1007/s00442-019-04435-8>
- Wandeler, H., and Bacher, S. (2006). Insect-transmitted urediniospores of the rust *Puccinia punctiformis* cause systemic infections in established *Cirsium arvense* plants. *Phytopathology* 96: 813–818. doi: 10.1094/PHYTO-96-0813
- Whitehead, A. G., & Hemming, J. R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of applied Biology*, 55(1), 25-38.
- Wilson, C.L. (1969). Use of plant pathogens in weed control. *Annual Review of Phytopathology* 7:411-434.
- Wilson, R. G. (1981). Effect of Canada thistle (*Cirsium arvense*) residue on growth of some crops. *Weed Science*, 29(2), 159-164.
- Zwolfer, H., & Harris, P. (1966). *Ceutorhynchus litura* (F.) (Col. Curculionidae), a potential insect for the biological control of thistle, *Cirsium arvense* (L.) Scop. in Canada. *Canadian Journal of Zoology*, 44(1), 3–38.

APPENDICES

APPENDIX A

PATHOLOGY OF CANADA THISTLE

Nucleotide sequences of different isolates

Bio-02

CCCTGTGACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACG
 GCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACCTTCTGAGTAAAACCATAAATAA
 ATCAAAACTTTCAACAACGGATCTCTTGTTTCTGGCATCGATGAAGAACGCAGCAAAATGCG
 ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCG
 CCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGT
 GGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGT
 AGTAGTAAAACCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAA
 TGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA
[MT579855.1](#)

Bio04

ACCCCTGTGACNTACCTTACTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACG
 GCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACCTTCTGAGTAAAACCATAAATAA
 ATCAAAACTTTCAACAACGGATCTCTTGTTTCTGGCATCGATGAAGAACGCAGCAAAATGCG
 ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCG
 CCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCCCTCGGGTTTGGT
 GTTGGGGATCGGCGAGCCTTTCTGGCAAGCCGGCCCCGAAATCTAGTGGCGGTCTCGCTGCA
 GCCTCCATTGCGTAGTAGTAAAACCTCGCAACTGGAACGCGGCGCGGCCAAGCCGTTAAA
 CCCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATC
[AMG820754.1](#)

Bio-05

TCNNACCCCTGTGAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGG
 GACGGCCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACCTTCTGAGTAAAACCATAA
 ATAAATCAAAACTTTCAACAACGGATCTCTTGTTTCTGGCATCGATGAAGAACGCAGCAAAA
 TGCATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG
 CCCGCCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTG
 GTGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATA
 GCGTAGTAGTAAAACCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTC
 TGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGA
 GGAN
[MN995067.1](#)

Bio-15

CCCCTGTGACATACCACTTGTTGCCTCGGCGGATCACCCGCTCCCGGTAAAACGGGACGGCC
 CGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACCTTCTGAGTAAAACCATAAATAAATC
 AAAACTTTCAACAACGGA
 TCTCTTGTTTCTGGCATCGATGAAGAACGCANCAAAATGCGATAAGTAATGTGAATTGCAGA
 ATTCAGTGAATCATCGAA
 TCTTTGAACGCACATTGCGCCCCGCCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCA
 ACCCTCAAGCACAGCTT
 GGTGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCAT
 AGCGTANTAGTAAAACCTCGTTACTGGTAATCGTCGCGGCCACGCCNTTAAACCCCAACTT
 CTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGA
 GGAAA

[MT967361.1](#)

Bio-17a

TACCTTACTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGG
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 ACAACGGATCTCTTGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTG
 AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGG
 CGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCCCTCGGGTTTGGTGTGTTGGGGATCG
 GCGAGCCTTTCTGGCAAGCCGGCCCCGAAATCTAGTGGCGGTCTCGCTGCAGCCTCCATTGC
 GTAGTAGTAAAACCCTCGCAACTGGAACGCGGCGCGGCCAAGCCGTTAAACCCCCAACTTCT
 GAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAG
 GAN

[MT516323.1](#)

Bio-22

CCCCTGTGACATACCACTTGTGCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGC
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 CAAAACTTTCAACAACGGATCTCTTGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGAT
 AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCC
 AGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTG
 GGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTA
 GTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCCAACTTCTGAAT
 GTTGACCTCGNNNNAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGNA

[MT579855.1](#)

Bio-24

CCCTGTGACATACCTTACTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGC
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 CAAAACTTTCAACAACGGATCTCTTGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGAT
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 AGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCCCTCGGGTTTGGTGT
 TGGGGATCGGCGAGCCTTTCTGGCAAGCCGGCCCCGAAATCTAGTGGCGGTCTCGCTGCAGC
 CTCCATTGCGTAGTAGTAAAACCCTCGCAACTGGAACGCGGCGCGGCCAAGCCGTTAAACCC
 CCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAAT
 AAGCGGAGGA

[PP345583.1](#)

Bio-29

CCCTGTGACATACCTTACTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGC
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 CAAAACTTTCAACAACGGATCTCTTGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGAT
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 AGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCCCTCGGGTTTGGTGT
 TGGGGATCGGCGAGCCTTTCTGGCAAGCCGGCCCCGAAATCTAGTGGCGGTCTCGCTGCAGC
 CTCCATTGCGTAGTAGTAAAACCCTCGCAACTGGAACGCGGCGCGGCCAAGCCGTTAAACCC
 CCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAAT
 AAGCGGAGGAN

[PP345583.1](#)

Bio-42

CCCTGTGAACATAACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGC
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 AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCC
 AGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGTTGTTG
 GGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTA
 GTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAAT
 GTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAANCGGAGGAN

[MN995067.1](#)

Bio-46

TCCCAACCCCTGTGACATAACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGG
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 ATAAATCAAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAA
 TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG
 CCCGCCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTG
 GTGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATA
 GCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTC
 TGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATC

[MT151384.1](#)

Bio-82

TCCCAACCCCTGTGACNTACCTTACTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACG
 GGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACCTTCTGAGTAAAACCATA
 AATAAATCAAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAA
 ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC
 GCCCGCCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCCCTCGGG
 TTTGGTGTGTTGGGGATCGGCGAGCCTTTCTGGCAAGCCGGCCCCGAAATCTAGTGGCGGTCTC
 GCTGCAGCCTCCATTGCGTAGTAGTAAAACCCTCGCAACTGGAACGCGGCGCGGCCAAGCC
 GTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAG
 CATATCA

[MG820754.1](#)

APPENDIX B

EFFICACY AND CROP SAFETY ASSESSMENT OF FUSARIA
SPECIES

a. Lutz farm 2023

Table A2.1. Crop emergence in Lutz farm for the year 2023

Isolates	Crop Emergence					
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower
Bio-02	120.38 ± 6.27	111.25 ± 8.05	68.88 ± 3.26	4.13 ± 1.05	64.88 ± 4.14	101.13 ± 33.67
Bio-04	118.25 ± 4.62	95.38 ± 5.75	60.63 ± 2.48	4.25 ± 1.11	53.75 ± 2.35	138.00 ± 1.34
Bio-05	125.88 ± 5.30	119.88 ± 4.71	61.88 ± 3.07	37.00 ± 32.67	62.50 ± 3.11	122.88 ± 16.90
Bio-15	123.25 ± 5.54	114.13 ± 5.93	68.38 ± 2.83	4.63 ± 0.66	59.25 ± 2.49	126.13 ± 10.04
Bio-29	130.00 ± 6.10	118.88 ± 6.04	59.50 ± 2.88	5.25 ± 2.05	58.88 ± 2.54	132.25 ± 7.07
Bio-82	122.63 ± 4.10	125.00 ± 2.72	55.75 ± 8.99	3.63 ± 1.42	54.63 ± 1.68	135.13 ± 6.31
Control	121.63 ± 4.57	109.38 ± 5.76	57.25 ± 7.67	6.63 ± 3.25	58.50 ± 1.47	127.00 ± 9.39
F-value	0.950					
P-value	0.546					

Table A2.2. Crop vigor in Lutz farm for the year 2023

Isolates	Crop Vigor					
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower
Bio-02	80.00 $\pm$ 0.00	78.75 $\pm$ 1.25	81.25 $\pm$ 1.25	68.75 $\pm$ 1.25	82.50 $\pm$ 1.44	67.50 $\pm$ 1.44
Bio-04	80.00 $\pm$ 0.00	80.00 $\pm$ 0.00	80.00 $\pm$ 0.00	70.00 $\pm$ 0.00	80.00 $\pm$ 0.00	70.00 $\pm$ 2.04
Bio-05	80.00 $\pm$ 0.00	80.00 $\pm$ 0.00	80.00 $\pm$ 2.04	70.00 $\pm$ 0.00	80.00 $\pm$ 0.00	67.50 $\pm$ 1.44
Bio-15	80.00 $\pm$ 0.00	77.50 $\pm$ 1.44	80.00 $\pm$ 2.04	68.75 $\pm$ 1.25	80.00 $\pm$ 0.00	68.75 $\pm$ 1.25
Bio-29	80.00 $\pm$ 0.00	78.75 $\pm$ 1.25	80.00 $\pm$ 2.04	68.75 $\pm$ 1.25	81.25 $\pm$ 1.25	67.50 $\pm$ 1.44
Bio-82	80.00 $\pm$ 0.00	78.75 $\pm$ 1.25	76.25 $\pm$ 1.25	63.75 $\pm$ 4.73	81.25 $\pm$ 1.25	66.25 $\pm$ 2.39
Control	80.00 $\pm$ 0.00	78.75 $\pm$ 1.25	76.25 $\pm$ 2.39	71.25 $\pm$ 1.25	80.00 $\pm$ 0.00	68.75 $\pm$ 1.25
F-value	1.091					
P-value	0.358					

Table A2.3. Crop height in Lutz farm for the year 2023

Isolates	Plant height (cms)					
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower
Bio-02	52.07 ± 2.46	80.85 ± 1.85	32.81 ± 0.94	24.13 ± 1.32	69.42 ± 1.73	46.99 ± 8.69
Bio-04	54.21 ± 2.06	81.10 ± 3.12	33.86 ± 1.09	25.61 ± 3.10	59.49 ± 2.51	56.32 ± 3.84
Bio-05	52.91 ± 0.43	83.82 ± 2.26	32.59 ± 1.02	38.53 ± 8.94	62.42 ± 1.91	55.04 ± 2.57
Bio-15	54.41 ± 1.75	82.73 ± 4.88	33.02 ± 0.91	28.58 ± 1.80	60.96 ± 1.63	56.97 ± 4.04
Bio-29	53.34 ± 0.91	79.38 ± 2.34	31.95 ± 1.88	25.61 ± 1.75	65.60 ± 5.11	56.08 ± 1.93
Bio-82	53.14 ± 2.31	81.73 ± 1.78	30.68 ± 1.12	27.51 ± 2.77	61.38 ± 2.24	52.26 ± 3.81
UTC	49.74 ± 1.88	84.02 ± 2.03	31.75 ± 0.56	26.87 ± 3.66	64.34 ± 2.84	56.09 ± 2.36
F-values	1.101					
P-value	0.346					

Table A2.4. Crop yield in Lutz farm for the year 2023

Isolates	Yield (g)		
	Wheat	Barley	Safflower
Bio-02	1403.50 ± 249.73	1800.68 ± 245.65	707.28 ± 222.79
Bio-04	1347.35 ± 107.84	1897.40 ± 380.75	885.28 ± 49.16
Bio-05	1246.50 ± 119.12	1783.33 ± 205.75	802.70 ± 42.29
Bio-15	1013.15 ± 300.55	1766.83 ± 267.44	787.50 ± 61.45
Bio-29	1249.28 ± 149.59	1734.30 ± 186.79	763.33 ± 111.45
Bio-82	1280.20 ± 80.75	1377.70 ± 437.31	919.18 ± 200.65
UTC	1302.43 ± 243.82	1674.58 ± 135.39	782.93 ± 55.99
F-values	0.884		
P-value	0.598		

b. Fort Ellis-2023

Table A2.5. Crop emergence in Fort Ellis farm for the year 2023

Isolates	Crop Emergence					
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower
Bio-02	96.88 ± 3.95	74.00 ± 9.52 bc	82.63 ± 6.46	15.63 ± 1.48	53.38 ± 0.43	125.25 ± 12.58
Bio-04	91.88 ± 7.52	63.88 ± 8.15 c	72.50 ± 1.86	16.38 ± 3.82	51.63 ± 1.60	130.25 ± 4.05
Bio-05	111.50 ± 3.24	100.13 ± 8.45 ab	77.13 ± 7.49	18.25 ± 1.96	53.13 ± 1.42	130.13 ± 12.58
Bio-15	110.25 ± 6.45	110.25 ± 7.27 a	86.50 ± 3.08	16.00 ± 2.11	53.38 ± 1.68	117.00 ± 12.25
Bio-29	103.00 ± 4.50	104.75 ± 4.68 ab	80.63 ± 4.86	18.13 ± 1.78	52.75 ± 1.83	108.25 ± 9.66
Bio-82	100.50 ± 7.60	96.75 ± 6.11 abc	76.75 ± 5.91	17.00 ± 2.97	53.00 ± 1.86	122.00 ± 8.50
UTC	86.13 ± 3.34	89.25 ± 6.86 abc	84.00 ± 5.92	19.38 ± 2.18	53.63 ± 1.05	114.38 ± 3.83
F-values	1.949					
P-value	0.006					

Table A2.6. Crop vigor in Fort Ellis farm for the year 2023

Isolates	Vigor					
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower
Bio-02	82.50 ± 1.44	78.75 ± 1.25	76.25 ± 1.25	83.75 ± 1.25	82.50 ± 1.44	82.50 ± 1.44
Bio-04	82.50 ± 1.44	80.00 ± 0.00	75.00 ± 0.00	81.25 ± 1.25	80.00 ± 0.00	81.25 ± 1.25
Bio-05	83.75 ± 1.25	82.50 ± 1.44	73.75 ± 1.25	82.50 ± 1.44	80.00 ± 0.00	80.00 ± 0.00
Bio-15	81.25 ± 2.39	81.25 ± 1.25	76.25 ± 1.25	82.50 ± 1.44	80.00 ± 0.00	82.50 ± 1.44
Bio-29	82.50 ± 1.44	81.25 ± 1.25	75.00 ± 2.04	82.50 ± 1.44	81.25 ± 1.25	81.25 ± 1.25
Bio-82	83.75 ± 1.25	80.00 ± 0.00	73.75 ± 1.25	80.00 ± 0.00	81.25 ± 1.25	80.00 ± 2.04
UTC	82.50 ± 1.44	80.00 ± 0.00	77.50 ± 1.44	82.50 ± 1.44	80.00 ± 0.00	82.50 ± 1.44
F-values	0.700					
P-value	0.871					

Table A2.7. Crop height in Fort Ellis farm for the year 2023

Isolates	Crop height					
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower
Bio-02	25.25 ± 1.39	33.33 ± 1.31	12.83 ± 0.69	21.42 ± 0.34	26.33 ± 2.20	25.00 ± 1.52
Bio-04	25.67 ± 0.41	33.92 ± 2.20	12.17 ± 0.40	21.33 ± 0.59	27.17 ± 1.60	23.58 ± 0.32
Bio-05	25.00 ± 0.24	37.08 ± 0.46	13.08 ± 0.72	21.42 ± 0.80	26.83 ± 0.42	23.83 ± 0.40
Bio-15	24.42 ± 0.60	35.75 ± 0.60	13.42 ± 0.37	20.67 ± 0.73	26.08 ± 2.28	24.42 ± 0.83
Bio-29	25.50 ± 0.22	35.00 ± 0.45	11.83 ± 0.79	22.08 ± 0.60	27.42 ± 0.76	24.33 ± 0.54
Bio-82	25.50 ± 0.52	35.00 ± 0.93	13.00 ± 1.05	19.75 ± 0.58	28.67 ± 0.36	23.25 ± 1.17
UTC	24.08 ± 0.82	35.17 ± 0.52	13.50 ± 0.29	21.50 ± 0.52	27.75 ± 0.76	24.42 ± 1.44
F-values	0.831					
P-value	0.716					

Table A2.8. Crop yield in Fort Ellis farm for the year 2023

Isolates	Yield (g)			
	Wheat	Barley	Pea	Safflower
Bio-02	1732.18 $\pm$ 162.89	868.48 $\pm$ 209.12	120.20 $\pm$ 19.50	1168.68 $\pm$ 72.73
Bio-04	1932.88 $\pm$ 141.33	1007.70 $\pm$ 181.36	141.23 $\pm$ 16.97	1003.75 $\pm$ 144.73
Bio-05	2027.88 $\pm$ 31.43	1187.65 $\pm$ 184.19	111.35 $\pm$ 16.03	1249.58 $\pm$ 43.73
Bio-15	2043.65 $\pm$ 21.69	1249.75 $\pm$ 162.76	112.33 $\pm$ 18.10	1068.75 $\pm$ 137.41
Bio-29	1797.85 $\pm$ 90.26	1174.88 $\pm$ 254.17	108.70 $\pm$ 6.68	1101.60 $\pm$ 113.66
Bio-82	1793.08 $\pm$ 108.93	1183.78 $\pm$ 72.62	117.18 $\pm$ 5.99	1185.00 $\pm$ 95.90
UTC	1500.50 $\pm$ 202.97	1217.35 $\pm$ 138.02	124.55 $\pm$ 14.32	1276.20 $\pm$ 88.66
F-values	1.253			
P-value	0.242			

c. Lutz Year-2024

Table A2.9. Crop emergence in Lutz farm for the year 2024

Isolates	Crop Emergence				
	Wheat	Barley	Lentil	Pea	Safflower
Bio-02	59.00 ± 7.33	64.00 ± 9.79	57.75 ± 1.93	52.00 ± 5.43	20.75 ± 6.43 c
Bio-04	66.50 ± 3.28	61.25 ± 6.79	71.25 ± 1.55	36.00 ± 2.86	33.75 ± 4.96 bc
Bio-05	71.25 ± 2.17	62.75 ± 9.83	72.50 ± 7.88	51.00 ± 2.65	64.25 ± 14.04 a
Bio-15	75.00 ± 9.50	63.25 ± 1.31	65.75 ± 3.68	47.00 ± 3.54	55.00 ± 13.03 abc
Bio-29	62.75 ± 4.53	74.50 ± 3.59	66.75 ± 4.57	43.75 ± 1.65	50.75 ± 10.16 abc
Bio-42	74.00 ± 7.58	72.25 ± 8.78	69.50 ± 3.33	42.00 ± 0.41	27.75 ± 7.65 bc
Bio-82	67.00 ± 7.22	57.25 ± 3.20	61.25 ± 4.23	40.50 ± 7.86	54.75 ± 5.12 ab
UTC	62.67 ± 5.61	61.00 ± 4.53	72.00 ± 6.75	44.40 ± 4.04	43.75 ± 2.25 abc
F-values	2.165				
P-value	0.002				

Table A2.10. Crop vigor in Lutz farm for the year 2024

Isolates	Crop Vigor				
	Wheat	Barley	Lentil	Pea	Safflower
Bio-02	76.25 $\pm$ 2.39	71.25 $\pm$ 2.39	77.50 $\pm$ 1.44	80.00 $\pm$ 0.00	71.25 $\pm$ 3.15
Bio-04	78.75 $\pm$ 1.25	72.50 $\pm$ 1.44	80.00 $\pm$ 0.00	78.75 $\pm$ 1.25	76.25 $\pm$ 2.39
Bio-05	77.50 $\pm$ 2.50	71.25 $\pm$ 2.39	80.00 $\pm$ 0.00	77.50 $\pm$ 1.44	80.00 $\pm$ 0.00
Bio-15	76.25 $\pm$ 1.25	75.00 $\pm$ 0.00	77.50 $\pm$ 1.44	76.25 $\pm$ 1.25	76.25 $\pm$ 2.39
Bio-29	75.00 $\pm$ 3.54	72.50 $\pm$ 3.23	78.75 $\pm$ 1.25	76.25 $\pm$ 1.25	73.75 $\pm$ 2.39
Bio-42	75.00 $\pm$ 2.04	72.50 $\pm$ 3.23	77.50 $\pm$ 2.50	78.75 $\pm$ 1.25	71.25 $\pm$ 3.15
Bio-82	77.50 $\pm$ 1.44	72.50 $\pm$ 1.44	76.25 $\pm$ 2.39	73.75 $\pm$ 1.25	80.00 $\pm$ 0.00
UTC	80.00 $\pm$ 0.00	72.50 $\pm$ 3.23	77.50 $\pm$ 2.50	75.00 $\pm$ 1.58	75.00 $\pm$ 2.04
F-values	2.127				
P-value	0.312				

Table A2.11. Crop height in Lutz farm for the year 2024

Isolates	Crop height				
	Wheat	Barley	Lentil	Pea	Safflower
Bio-02	55.25 ± 1.46	55.67 ± 2.70	29.42 ± 1.14	44.17 ± 3.80	39.75 ± 3.13
Bio-04	57.75 ± 1.76	58.92 ± 1.55	29.17 ± 0.70	39.08 ± 1.26	40.50 ± 3.41
Bio-05	57.75 ± 3.19	55.42 ± 2.14	29.92 ± 1.57	42.67 ± 1.87	48.50 ± 1.04
Bio-15	57.00 ± 1.93	58.75 ± 0.25	26.67 ± 1.97	42.92 ± 2.57	41.58 ± 1.70
Bio-29	54.00 ± 3.01	60.92 ± 4.57	29.83 ± 1.36	40.42 ± 1.49	41.42 ± 2.51
Bio-42	54.92 ± 2.73	58.50 ± 1.71	29.75 ± 0.91	42.58 ± 1.82	37.08 ± 2.05
Bio-82	56.75 ± 1.72	58.58 ± 1.72	28.25 ± 1.23	36.83 ± 1.65	44.92 ± 1.31
UTC	59.22 ± 2.63	62.25 ± 2.50	29.17 ± 0.50	38.27 ± 1.80	42.25 ± 2.56
F-values	1.443				
P-value	0.0913				

Table A2.12. Crop yield in Lutz farm for the year 2024

Isolates	Yield (g)				
	Wheat	Barley	Lentil	Pea	Safflower
Bio-02	791.03 ± 19.51	673.95 ± 176.63	531.08 ± 60.41	407.20 ± 44.99	108.78 ± 11.93
Bio-04	876.03 ± 58.20	910.93 ± 170.52	478.50 ± 47.76	344.73 ± 60.76	131.33 ± 23.60
Bio-05	1046.68 ± 178.86	830.85 ± 226.99	589.98 ± 52.69	454.60 ± 81.72	166.50 ± 12.55
Bio-15	861.50 ± 152.55	1024.60 ± 189.28	485.20 ± 122.53	457.18 ± 54.70	127.88 ± 6.96
Bio-29	712.70 ± 189.17	924.68 ± 47.79	615.13 ± 69.39	408.53 ± 62.92	129.78 ± 18.99
Bio-42	858.20 ± 118.93	942.25 ± 199.26	489.60 ± 59.13	557.25 ± 97.89	107.00 ± 8.40
Bio-82	960.25 ± 159.99	979.00 ± 153.83	492.33 ± 74.09	397.45 ± 52.94	133.90 ± 7.43
UTC	1095.10 ± 326.81	913.68 ± 210.83	552.75 ± 82.47	550.38 ± 64.53	116.25 ± 10.10
F-values	0.678				
P-value	0.883				

d. Fort Ellis- 2024

Table A2.13. Crop emergence in Fort Ellis farm for the year 2024

Isolates	Crop Emergence					
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower
Bio-02	69.00 $\pm$ 3.67	72.00 $\pm$ 7.47	74.00 $\pm$ 3.03	16.00 $\pm$ 5.82	48.50 $\pm$ 3.80	72.33 $\pm$ 13.04
Bio-04	71.25 $\pm$ 2.29	66.50 $\pm$ 7.90	73.00 $\pm$ 2.61	23.50 $\pm$ 4.05	52.75 $\pm$ 1.44	66.25 $\pm$ 10.06
Bio-05	79.50 $\pm$ 4.41	63.00 $\pm$ 1.47	65.25 $\pm$ 5.56	14.00 $\pm$ 11.03	49.75 $\pm$ 2.10	75.00 $\pm$ 8.58
Bio-15	72.75 $\pm$ 6.02	70.50 $\pm$ 4.37	70.75 $\pm$ 6.49	15.75 $\pm$ 6.55	45.00 $\pm$ 2.16	73.25 $\pm$ 8.94
Bio-29	70.50 $\pm$ 2.40	73.25 $\pm$ 5.95	80.25 $\pm$ 6.98	19.50 $\pm$ 3.57	47.00 $\pm$ 6.12	83.25 $\pm$ 5.11
Bio-42	67.75 $\pm$ 4.66	67.75 $\pm$ 8.01	66.50 $\pm$ 3.12	17.25 $\pm$ 3.35	47.50 $\pm$ 0.50	68.75 $\pm$ 6.56
Bio-82	59.25 $\pm$ 2.53	70.25 $\pm$ 5.36	60.25 $\pm$ 7.00	15.33 $\pm$ 7.22	42.50 $\pm$ 3.59	77.00 $\pm$ 11.03
UTC	78.00 $\pm$ 4.00	86.25 $\pm$ 5.42	65.50 $\pm$ 1.66	11.33 $\pm$ 0.67	49.40 $\pm$ 5.72	59.25 $\pm$ 5.65
F-values	1.026					
P-value	0.441					

Table A2.14. Crop vigor in Fort Ellis farm for the year 2024

Isolates	Crop Vigor					
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower
Bio-02	75.00 $\pm$ 2.04	73.75 $\pm$ 3.15	78.75 $\pm$ 1.25	75.00 $\pm$ 0.00	77.50 $\pm$ 1.44	73.33 $\pm$ 1.67
Bio-04	76.25 $\pm$ 2.39	72.50 $\pm$ 3.23	77.50 $\pm$ 2.50	75.00 $\pm$ 0.00	78.75 $\pm$ 1.25	75.00 $\pm$ 2.04
Bio-05	78.75 $\pm$ 1.25	73.75 $\pm$ 2.39	78.75 $\pm$ 1.25	76.67 $\pm$ 1.67	77.50 $\pm$ 1.44	76.25 $\pm$ 1.25
Bio-15	76.25 $\pm$ 1.25	73.75 $\pm$ 2.39	76.25 $\pm$ 2.39	73.75 $\pm$ 1.25	75.00 $\pm$ 0.00	72.50 $\pm$ 3.23
Bio-29	75.00 $\pm$ 2.89	72.50 $\pm$ 1.44	78.75 $\pm$ 1.25	75.00 $\pm$ 0.00	77.50 $\pm$ 1.44	73.75 $\pm$ 1.25
Bio-42	77.50 $\pm$ 2.50	72.50 $\pm$ 4.33	76.25 $\pm$ 1.25	76.25 $\pm$ 1.25	78.75 $\pm$ 1.25	75.00 $\pm$ 0.00
Bio-82	76.25 $\pm$ 2.39	70.00 $\pm$ 2.89	77.50 $\pm$ 1.44	76.67 $\pm$ 1.67	76.25 $\pm$ 1.25	73.75 $\pm$ 1.25
UTC	77.50 $\pm$ 1.44	71.25 $\pm$ 3.75	77.50 $\pm$ 1.44	75.00 $\pm$ 0.00	78.75 $\pm$ 1.25	72.50 $\pm$ 1.44
F-values	0.519					
P-value	0.987					

Table A2.15. Crop height in Fort Ellis farm for the year 2024

Isolates	Crop height					
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower
Bio-02	58.58 ± 3.44	69.25 ± 2.74	31.17 ± 0.89	43.25 ± 2.23	50.92 ± 2.63	32.56 ± 3.80
Bio-04	59.75 ± 3.39	62.67 ± 3.73	31.75 ± 1.41	44.33 ± 1.01	50.67 ± 3.11	35.75 ± 1.16
Bio-05	62.25 ± 1.80	71.92 ± 2.31	32.08 ± 1.64	42.22 ± 6.07	47.25 ± 2.06	38.50 ± 2.21
Bio-15	52.17 ± 7.75	64.67 ± 3.31	31.75 ± 0.32	34.42 ± 7.64	43.08 ± 3.25	40.08 ± 2.67
Bio-29	60.25 ± 3.64	68.17 ± 2.47	31.67 ± 1.57	42.75 ± 1.39	50.83 ± 2.15	36.75 ± 1.62
Bio-42	63.42 ± 2.78	67.25 ± 5.18	31.50 ± 0.35	37.83 ± 3.26	50.08 ± 2.47	37.67 ± 1.96
Bio-82	54.58 ± 1.94	62.67 ± 3.09	30.58 ± 1.36	43.78 ± 0.87	46.75 ± 4.12	35.75 ± 2.36
UTC	60.25 ± 2.14	70.92 ± 5.97	32.42 ± 1.31	39.00 ± 2.65	48.33 ± 1.59	36.67 ± 4.17
F-values	0.923					
P-value	0.595					

Table A2.16. Crop yield in Fort Ellis farm for the year 2024

Isolates	Yield (g)					
	Wheat	Barley	Lentil	Chickpea	Pea	Safflower
Bio-02	957.18 ±	374.23 ±	518.08 ±	289.45 ±	383.75 ±	452.60 ±
	234.87	139.90	49.49	110.02	37.14	106.68
Bio-04	1120.80 ±	309.15 ±	527.50 ±		434.45 ±	134.90 ±
	288.98	54.17	81.11	424.77 ± 19.50	112.27	18.07
Bio-05	1067.28 ±	351.08 ±	441.15 ±	462.33 ±	406.88 ±	166.48 ±
	156.09	70.53	45.78	227.73	99.21	24.01
Bio-15	1352.27 ±	339.38 ±	533.83 ±		374.48 ±	116.50 ±
	198.73	106.39	109.58	319.13 ± 85.17	74.51	12.44
Bio-29	986.65 ±	419.25 ±	557.83 ±		438.10 ±	138.90 ±
	216.57	148.33	98.73	341.13 ± 69.94	66.15	21.06
Bio-42	1011.60 ±	309.58 ±	486.00 ±		395.55 ±	173.55 ±
	159.41	66.88	67.31	338.73 ± 47.36	77.92	44.31
Bio-82	887.58 ±	287.40 ±	448.60 ±	546.15 ±	328.23 ±	152.28 ±
	204.37	62.41	39.22	190.95	28.63	14.88
UTC	1331.25 ±	378.88 ±	568.60 ±		452.60 ±	129.33 ±
	100.57	100.23	124.54	175.93 ± 24.84	106.68	7.24
F-values	0.578					
P-value	0.969					