Biology and Interaction of the Invasive Forb *Isatis tinctoria*, Dyer's Woad, with the Native Rust Fungus *Puccinia thlaspeos* in Populations in the Intermountain West, United States

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Authorization to Submit Thesis

This thesis of Robert D. Gibson II, submitted for the Degree of Master of Science with a Major in Environmental Science and titled "Biology and Interaction of the Invasive Forb *Isatis tinctoria*, Dyer's Woad, with the Native Rust Fungus *Puccinia thlaspeos* in Populations in the Intermountain West, United States," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

Isatis tinctoria (Brassicaceae), dyer's woad, a plant of Eurasian origin, has become invasive after its introduction to western North America. Traits that may contribute to its invasiveness include prolific seed production, a rapid growth rate, an extensive two-layered rooting pattern, high phenotypic plasticity, and assumed allelopathic properties. Integrated weed management methods have shown some success, but currently *I. tinctoria* is not considered controlled. *Puccinia thlaspeos* 'woad strain,' a rust fungus native to North America, was discovered on *I. tinctoria* and later developed as a registered mycoherbicide. We quantified the presence and incidence of *P. thlaspeos* 'woad strain' in natural populations and its effect on the reproductive output of *I. tinctoria* at the individual plant and population level, and on a larger geographical scale. *P. thlaspeos* 'woad strain' may have synergistic effects when combined with other management practices and/or potential classical biological weed control organisms currently studied in Europe.

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Dedication

I dedicate this thesis to my friends and family members as a reference of encouragement and a reminder that we can all accomplish anything we put our minds to with the love and support of genuine, honest people!

I warmly thank the Vandal Marching Band, Vandal Cheer Squad, Joe Vandal, and my friends and family members for their patience, understanding, infinite encouragement, project management, and love through this life changing experience.

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Name

Isatis tinctoria L. (dyer's woad), from which the modern term 'weed' was derived, is commonly referred to as 'woad' or dyeweed (UK) (King, 1966), or Marlahan mustard in northern California (Grantham, 2015). Derivatives of 'woad' include 'wād' (Old English), 'waide' (French), 'waid' (German), and 'añil' (Spanish) (Hurry, 1930; Sales et al., 2006; Varga and Evans, 1978). 'Woad' is not only a common name for *I. tinctoria*, but it also refers to the indigo dye extracted from the plant (woad dye) (Hurry, 1930). Historical names for *I. tinctoria* were 'vitrum' or 'glastum', the Latin translation and Latinization, respectively, of the Celtic word 'glas' meaning glass or blue (Guarino et al., 2000; King, 1966). The historical names were commonly used to identify specific variants: cultivated or garden woad (*Glastum sativum, Glasto domestico*), and wild woad (*Glastum sylvestris, Isatis sylvestris, Glasto sylvatico*) (Hurry, 1930).

I. tinctoria is a member of the family Brassicaceae (Cruciferae), a monophyletic group characterized by a cruciform floral arrangement, six stamens per flower (two short and four long), and distinctive fruit capsules (siliques or silicles) (Beilstein et al., 2006; Hitchcock and Cronquist, 1973; Hurry, 1930). The family Brassicaceae consists of 49 tribes, 321 genera, and 3,660 species worldwide with extensive homoplasy occurring in almost every morphological character (Al-Shehbaz, 2012). Morphological characters were used to delineate the family taxa prior to molecular phylogenetic studies (Al-Shehbaz et al., 2006); however, classification based on the use of the internal transcribed space (ITS) (Bailey et al., 2006; Moazzeni et al., 2010; Warwick et al., 2010), chloroplast gene *ndh*F (Beilstein et al., 2006; Beilstein et al., 2008), phytochrome A (*phyA*) (Beilstein et al., 2008), mitochondrial markers (*nad4* intron 1) (Couvreur et al., 2010) and trichome morphology (Beilstein et al., 2006) has resulted in recognition of three main lineages. *I. tinctoria* is within lineage II and a member of the tribe Isatideae (Beilstein et al., 2006). The tribes that comprise lineage II are primarily glabrous or bear simple trichomes (Al-Shehbaz, 2012). Isatideae consists of ca. 5 monophyletic genera (ca. 90 species) all of which are primarily distributed in central and southwestern Asia, with fewer species in Europe, and no species native to North America (Al-

Shehbaz, 2012). The species within the tribe Isatideae are characterized by yellow or rarely white flowers, 1- or 2-seeded indehiscent fruits, and often auriculate cauline leaves (Al-Shehbaz et al., 2006). The genus *Isatis* (ca. 86 spp.) is distinguished by the flattened, indehiscent, and usually one seeded, winged fruits (Al-Shehbaz, 2012). *I. tinctoria* is one of the most widely distributed species in the genus (King, 1966).

Description and account of variation

Species description

The following description is primarily based on the following taxonomic accounts Callihan (1990), Callihan et al. (1984), Davis (1985), Evans (1991), Farah et al. (1988), Guarino et al. (2000), Hitchcock and Cronquist (1964), Hitchcock and Cronquist (1973), Hurry (1930), McConnell et al. (1999), Moazzeni and Zarre (2007), and Varga and Evans (1978).

I. tinctoria is a winter annual, biennial, or short-lived herbaceous perennial with a two-layered rooting pattern: taproot and lateral roots. The taproot length varies among regions, from 0.30 m to 0.46 m in Europe and from 1.0 m to 2.0 m in North America, with additional variation dependent on soil moisture and type. Total root length (taproot and lateral branching) in North America ranges from 2.2 m to 2.6 m, while perennial plants can have more extensive root systems.

The leaves are waxy and slightly pubescent, nearly entire, and glaucous (bluish-green) with a thick silvery mid-vein, commonly used as an identifying character. Both radical and aerial leaves are seen. Radical (basal) leaves are oblong to lanceolate or ovate, widest near the tip, ranging from 3.2 cm to 17.8 cm, up to 30 cm, and they are connected to the root crown (rosette stage) or occasionally the base of the stem (flowering stage) via a petiole (0.5 to 5.5 cm). Aerial (cauline) leaves are sessile, oblong-lanceolate to lanceolate, and sagittate or auriculate at the base with an acute apex. Cotyledons are obtuse, oblong, and entire.

Stems are glaucous, usually glabrous and paniculately branched above the middle, and range from 0.3 m to 1.5 m in length. Aerial leaves develop alternately from half to three-quarters of the way up the stem, underneath the inflorescences. Inflorescences divide into panicles (compound racemes), forming an umbrella of yellow flowers. Individual flowers measure approximately 4 mm in diameter. Each flower consists of four oblong sepals

arranged in pairs at right angles and four petals arranged crosswise. The petals are narrowly oblanceolate and twice the length of the sepals (2.5 mm to 4.0 mm versus 1.5 mm to 2.8 mm, respectively). The six stamens are divided into two sets: a long outer pair and two short inner pairs (1 mm to 2.5 mm and 0.5 mm to 0.7 mm, respectively). As each flower matures, the carpel develops into a lime-green silicle (fruit or seedpod). The pendulous silicles hang freely from a slender, deflexed pedicel, subclavate at apex, and range from 6.4 mm to 12.7 mm in length. Mature silicles are glabrous, pendulous, oblong-teardrop (2.5 mm to 7 mm wide), and broad at the apex (8 mm to 18 mm long). They are winged all around with the apical wing 3.5 mm to 5 mm wide. The locule (central seed capsule) contains a distinct mid-vein and inconspicuous lateral vein. Each locule contains one cylindrical or oblong seed, 2.3 mm to 3.5 mm long, and yellowish light brown in color. In the rare instance two seeds are formed, one is usually non-viable. Upon maturity, the indehiscent silicles vary in color depending on the variant (e.g. cream, purple, brown, black). As the stem begins to lignify, silicles fade in color to pale yellow or cream, and slowly decompose.

The chromosome number for *I. tinctoria* is 2n = 14, 28, or 2n = 4x = 28. Accounts in central Asia reported 2n = 14, and two accounts in the British Isles reported 2n = 28 (Clapham et al., 1962; Hurry, 1930; Moazzeni and Zarre, 2007), while the chromosome atlas of flowering plants has reported 2n = 4x = 28, x=7 for *I. tinctoria* and all members of the tribe Isatideae (Al-Shehbaz et al., 2006; Darlington and Wylie, 1955; Spataro et al., 2007; Warwick and Al-Shehbaz, 2006).

Distinguishing features

I. tinctoria is easily recognized in North America because there are no *Isatis* species native to the continent (Al-Shehbaz, 2012; CWMA, 2016). The characters that distinguish the plant are 1) leaves with a silvery mid-vein, 2) two leaf forms, 3) erect habit and lignified stem, 4) showy umbrella-like floral presentation consisting of small, bright yellow flowers, and 5) dark colored silicles (Callihan et al., 1984; Callihan and Miller, n.d.; McConnell et al., 1999; Reeves, 2010; Jacobs and Pokorny, 2007).

In its native range, *I. tinctoria* superficially resembles three species endemic to Iran (*I. guabae* Bornm., *I. pachycarpa* Rech f., Aellen & Esfand., and *I. takhtajanii* Avestisian, Izv. Akad. Nauk Arm.) and *I. indigotica* Fort. (Chinese Woad). *I. guabae* can be distinguished by

its linear-oblong fruits with the locule wider than the wings; *I. pachycarpa* by its distinct pubescent leaves, velvety silicles, and carinate fruit locule; *I. takhtajanii* by its perennial habit, subobtuse auricle of aerial leaves, elliptic-oblong silicles shape, larger silicles size, and wider locule located in the middle to base of the silicles; and *I. indigotica* by its glabrous leaves and more erect stems (Moazzeni and Zarre, 2007; Moazzeni et al., 2008; Sales et al., 2006; Tozzi et al., 2005).

Intra-specific variation

The most recent taxonomic treatments of *I. tinctoria* recognize three subspecies: *I. tinctoria* subsp. *athoa* (Boiss.) Papan, subsp. *corymbosa* (Boiss.) P. H. Davis, and subsp. *tomentella* (Boiss. & Balansa) P. H. Davis, along with 33 synonyms (The Plant List, 2013). However, *I. tinctoria* subsp. *tomentella* has been speculated to be a form of *I. kotschyana* Boiss & Hohen. based on habit and the shape of its aerial leaves (Moazzeni et al., 2008).

I. tinctoria has never been subject to a formal breeding program. Nevertheless, the extensive cultivation of this plant across Europe led to the development of unique and locally adapted populations with distinct phenotypic characteristics (lineages) (Gilbert and Cooke, 2001; Gilbert et al., 2002; Guarino et al., 2000; Spataro and Negri, 2008a). *I. tinctoria* is highly polymorphic across Eurasia and Europe, 80.3% to 96.6% polymorphic bands (Gilbert et al., 2002; Rocha et al., 2011b; Spataro and Negri, 2008a; Spataro et al., 2007). Variation among lineages is morphologically based on fruit and seed shape and leaf forms (Hegi, 1986; Pignatti, 1982). Many lineages are recognized; however, names and/or precise distributions have not been recorded in detail (Turkmen et al., 2004). Recent analyses of phenotypic and genotypic information have shown that lineages can be distinguished predominantly by geographic origin and regional distribution (Rocha et al., 2011b; Spataro and Negri, 2008a; Spataro et al., 2007). There are two distinct gene pools recognized, Europe and Central Asia. These pools cannot be separated due to the 1) assumed Asian origin of *I. tinctoria* and 2) high polymorphism found within lineages and individual populations (41.0% to 73.1% polymorphic bands), with greater variation within lineages than among them (58% vs. 42%, polymorphic bands respectively) (Gilbert et al., 2002; Rocha et al., 2011b; Spataro and Negri, 2008a; Spataro et al., 2007). European lineages are better grouped than Central Asian lineages; the factors assumed responsible for grouping those lineages are the environmental

components and genetic drift (Spataro and Negri, 2008a; Spataro et al., 2007). In North America, all *I. tinctoria* populations are of a France Germany Italy Morocco Switzerland Ukraine genetic origin (J. Gaskin, pers. comm.).

Economic importance and environmental impact

Detrimental impacts

I. tinctoria invasions have had negative consequences in North America. *I. tinctoria* was not considered an invasive species until it was introduced into western North America during the early 20th century, where it naturalized rapidly and spread throughout the western states and provinces (Evans and Chase, 1981; King, 1967; Young and Evans, 1977). Between 1971 and 1981, the dispersal rate of *I. tinctoria* in Utah doubled and was estimated to cost the state $2 million in losses of agricultural and rangeland production in reduced forage quality of pastures and hay fields (Evans and Chase, 1981; Young, 1988). The spread of *I. tinctoria* on the United States Department of Interior (USDI) Bureau of Land Management lands in the Pacific Northwest increased at an estimated annual rate of 14% and the average cattle grazing capacity was reduced by 38% (United States Department of Interior, 1985; Jacobs and Pokorny, 2007; Young, 1988). On USDA Forest Service lands in the Intermountain Region, the number of infested hectares increased more than 35-fold between 1969 and 1985 (United States Department of Agriculture, 1986). Additionally, an infestation in Montana increased more than 50-fold, from 0.8 to 40.5 ha, in two years (Aspevig et al., 1985). While in the USDA Cache National Forest in northern Utah, 55 of the 60 land-cover types studied were invaded by *I. tinctoria*, and 16.6% or 24,802 ha of the area was estimated to be suitable to *I. tinctoria* invasion with a potential minimum increase of a 124-fold (Dewey et al., 1991).

I. tinctoria is considered an early successional species that exhibits high plasticity across heterogeneous environments, with the ability to thrive even in suboptimal and unfavorable environments (Monaco et al., 2005; Sonmez et al., 2008; Zouhar, 2009). It outcompetes desirable species (CISM, 2014; DiTomaso and Kyser, 2013). Traits that may aid its invasiveness include its prolific seed production (McConnell et al., 1999), a rapid growth rate (Jacobs and Pokorny, 2007), its two-layered rooting pattern (Farah et al., 1988), and assumed allelopathic properties. *I. tinctoria* solely disperses via seed and establishes well along transportation and movement corridors, in disturbed habitats, and even has the ability to

invade undisturbed habitats, both densely and sparsely vegetated (NWCB, 1999; Roché, 1992). Plants resume growth in early spring, less than a week after snowmelt, and begin to bolt about two weeks later (Farah, 1987; Jacobs and Pokorny, 2007). The two-layered rooting pattern, similar to that of *Artemisia tridentata* Nutt. (big sagebrush), allows *I. tinctoria* to effectively compete for resources and colonize habitats opportunistically (CISM, 2014; DiTomaso and Kyser, 2013; Farah, 1987; Monaco et al., 2005; Jacobs and Pokorny, 2007). In northern California, *I. tinctoria* is considered a threat to the survival of the endangered *Phlox hirsuta* E. E. Nelson (Yreka phlox) and *Calochortus persistens* Ownbey (Siskiyou mariposa lily (Diggles et al., 2004; Grantham, 2015). While there are currently no apparent adverse effects of scattered *I. tinctoria* populations on the survival of *P. hirsuta*, substantial infestations could pose a significant threat (FWS, 2006). However, *I. tinctoria* is a known threat to the survival and prevention of seedling establishment of *C. persistens* through its competition and allelopathic properties (FWS, 2011; Grantham, 2015). In 2003, extensive surveys determined *I. tinctoria* has infested 75.2% of the *C. persistens*' known habitat and up to 10% of that habitat was densely covered (FWS, 2012; Knapp, 1997; Klamath National Forest, 2005). Over the last 100 years, *I. tinctoria* has invaded most of western North America and is declared noxious in 11 western states (Hansen and Bloem, 2006).

I. tinctoria is known for its chemical properties (Mohn et al., 2009) (see also Section 7). The water-soluble chemical liquid (composition not identified) released from the silicles (seedpods) inhibits germination and root elongation of competitive species (Farah, 1987; Young and Evans, 1971). The water-soluble substance contributes to the formation of the *I. tinctoria* seed bank through minimizing intraspecific competition and delaying germination until environmental conditions are favorable (NWCB, 1999; Young and Evans, 1971). *I. tinctoria*'s chemical defense compounds make the plant unpalatable to livestock and native herbivores (NWCB, 1999).

Beneficial

The indigo dye industry in Europe was solely reliant on *I. tinctoria* (Gilbert and Cooke, 2001). Indigo is a product of plant secondary metabolites and is formed through the oxidation of damaged tissue when exposed to air (see Section 7) (Hancock, 1997; Stoker et al., 1998a). Indigo was extracted from *I. tinctoria* in a woad mill through the process of woad

balls (UK) or woad loaves (Italy), which is thoroughly described by Hurry (1930) and Guarino et al. (2000). In summary, harvested leaves were crushed into pulp and the pulp was then thrown into small heaps to drain. When dry, the pulp was hand kneaded into balls approximately 10 cm to 12 cm in diameter and dried. After one to four weeks, the woad balls were grounded into a powder and then heaped into a layer of 0.6 m to 1.0 m. The powder was sprinkled with water and allowed to ferment, creating a 'powdered paste'. The mass was watered and rotated frequently for approximately nine weeks. After fermentation, the mass converted to a dark clay-like substance that was dried, sifted, and packed tightly into wooden barrels for shipment to the dyers (Hurry, 1930). The use of indigo dye ranged from clothing (Berkeley, 1961; Guarino et al., 2000) to armor for war (Varga and Evans, 1978), to tattoos (Guarino et al., 2000; Spataro et al., 2007). Other dyes were commonly mixed with different shades of indigo to produce a range of colors; the shade of indigo was an indicator of wealth in society (Hurry, 1930).

In addition to its cultivation as a textile dye crop, *I. tinctoria* was valued for its medicinal properties (Hurry, 1930; Varga and Evans, 1978). The indole-derived compounds, glucosinolates specifically, located in the leaves, roots, and seeds contain anti-inflammatory, anti-carcinogen, antiallergenic, anti-arthritic, and anti-tumoral properties (Hamburger, 2002; Heinemann et al., 2004; Michnovicz and Bradlow, 1990; Oberthür et al., 2005; Recio et al., 2006a; Recio et al., 2006b). Active compounds, such as tryptanthrin, have been found to inhibit prostaglandin and leukotriene synthesis (Danz et al., 2002b; Danz et al., 2001; Oberthür and Hamburger, 2004; Recio et al., 2002). Indigo precursors and glucosinolates also exhibit anti-fungal properties (Ahmad and Fatima, 2008) and the essential oils extracted from the seeds are used as a source of oil and in the production of cosmetics (Spataro and Negri, 2008a). Other benefits of *I. tinctoria* have included the use as an ornamental decoration for garden borders (Hurry, 1930; NWCB, 1999), and prevention of erosion along hill slopes (Varga and Evans, 1978). *I. tinctoria* has the ability to accumulate and store heavy metals, such as lead, cadmium, and low concentrations of zinc without any apparent consequences to growth and reproduction (Sonmez et al., 2008).

Legislation

I. tinctoria is an exotic weed in 14 North American states, and declared noxious in 11 of those states and the province of British Columbia (Hansen and Bloem, 2006; USDA, 2016). These states are: Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming (Callihan and Miller, n.d.; USDA, 2010). Arizona, Colorado, New Mexico, and Washington categorize *I. tinctoria* as a 'Class A' noxious weed (i.e. prevent new outbreaks and eliminate existing ones) (CWMA, 2016; NWCB, 1999; Zouhar, 2009), the California Invasive Plant Council (Cal-IPC) declared the plant's invasiveness as moderate (DiTomaso and Kyser, 2013), and Montana downgraded it from a Category 2 to a Category 3 noxious weed (Jacobs and Pokorny, 2007). In contrast, under the crop diversification policy of the European Union, *I. tinctoria* is being re-introduced in Europe as a natural source of indigo dye to be grown in mountainous and marginal areas as an alternative to traditionally-grown crops (Spataro and Negri, 2008a).

Geographic distribution

I. tinctoria is globally the most widely distributed species in the *genus Isatis* (Hurry, 1930). The plant's current distribution spans most of the northern hemisphere (i.e. Asia, Europe, and North Africa, from the British Isles to China and Japan) and parts of North and South America (Callihan et al., 1984; King, 1967; King, 1966). The abundance of lineages throughout Europe and Asia makes it difficult to identify the center of origin and define the native range (Gilbert et al., 2002; Spataro and Negri, 2008a). Suggested evolutionary centers of origin include southeastern Asia (Sales et al., 2006), Asia or southeastern Europe (Pignatti, 1982), and the eastern Mediterranean and the Ponto region (i.e. northern Turkey along the coast line of the Black Sea) (Hegi, 1986). *I. tinctoria* was recorded as a native plant of the Russian steppes, growing on exposed hillsides (Dunn, 1905; Evans and Gunnell, 1982), while the native range is assumed to span the steppes and desert regions of the Caucasus Mountains to eastern Siberia and the Near East (Hegi, 1986; Jacobs and Pokorny, 2007). Recent molecular data confirms that *I. tinctoria* had an Asian center of origin and then spread west towards Europe (Spataro et al., 2007). The largest morphological diversity of *I. tinctoria* lineages in the northern hemisphere was found in Turkey and the eastern Mediterranean areas (Hegi, 1986)

I. tinctoria has a wide adventive range in Asia and Europe. In Asia, *I. tinctoria* has been reported in Afghanistan, Asia Minor, Iran, Japan, Kashmir, Kazakhstan, Korea, Mongolia, Pakistan, Russia, Tajikistan, Tibet, Turkey, and Uzbekistan (Hurry, 1930; King, 1966; Moazzeni and Zarre, 2007; Varga and Evans, 1978). At the peak of the indigo dye industry, the textile crop was distributed throughout most of Europe; however, records of its distribution decreased and became fragmented after cultivation ceased in the early 20th century (Hegi, 1986). Based on records of cultivation and indigo production, the adventive range included Austria, the Canary Islands, Corsica, France, Germany, Great Britain, Holland, central and southern Italy and its major islands, Macedonia, Madeira, Portugal, Spain, and Switzerland, along with Morocco and Egypt in North Africa (Gilbert and Cooke, 2001; Guarino et al., 2000; Hurry, 1930; Mohn and Hamburger, 2008; Rocha et al., 2011b; Sales et al., 2006; Stoker et al., 1998a; Young and Evans, 1977). In North America, *I. tinctoria* has a patchy distribution throughout Canada and the United States. In Canada, the plant has naturalized in Ontario and Quebec, and is persistent and spreading through British Columbia, including Vancouver Island (USDA, 2016). In the United States, *I. tinctoria* established and naturalized similarly in some eastern states (i.e. parts of Illinois, Maryland, Missouri, New Jersey, New York, Virginia, and West Virginia) (Callihan et al., 1984; Evans and Gunnell, 1982; USDA, 2010). West of the Missouri River, *I. tinctoria* is invasive in Arizona, California, Colorado, Idaho, Montana, New Mexico, Nevada, southern Oregon, Utah, Washington, and Wyoming (Callihan, 1990; Evans and Chase, 1981; Evans, 1991; Jacobs and Pokorny, 2007; McConnell et al., 1999; Robbins et al., 1951; USDA, 2010; USDA Forest Service, 2014).

Habitat

Climatic requirements

I. tinctoria is adapted to the temperate climates and is influenced by seasonality (King, 1966). Changes in temperature determine the onset of flowering with no effect on the duration of the ripening period (Spataro and Negri, 2008a). Plants successfully reproduce at elevations ranging from 380 m to 2700 m above mean sea level (AMSL) with plant mortality greatest at elevations below 900 m, assumedly caused by heat (Callihan, 1990; Spataro and Negri, 2008a). *I. tinctoria* is less tolerant to heat, extended periods of drought, and shade,

than to hard long winters below 0 °C with full sun (Monaco et al., 2005; Spataro and Negri, 2008a). The species prefers mesic or mesic-xeric conditions (adequate moisture through the season or abundant moisture early in the season with drier conditions following) (Lackschewitz, 1986; Booth and Wright, 1962). In the American Intermountain West, plants are commonly found on windy, south-facing canyon and hill slopes (Callihan et al., 1984; Holmgren, 1958; Roché, 1992).

Substratum

In Europe, *I. tinctoria* flourishes in a wide variety of soil types, from vineyards in Germany and Switzerland (H. Hinz, pers. comm.), to arid and rocky soils in Italy (Guarino et al., 2000), and from old lime pits and chalk quarries in England to deep, fertile, well-drained, loam or clay soils during cultivation (Hurry, 1930). In western North America, *I. tinctoria* often establishes in rocky or sandy alkaline, bench soils (Callihan, 1990; McConnell et al., 1999; Varga and Evans, 1978). Plants are hardy and tolerant, often found in wastelands and can withstand soils contaminated with heavy metals (Sonmez et al., 2008).

Communities in which the species occurs

The habitats most susceptible to invasion by *I. tinctoria* include south-facing hill slopes, farmlands and fallow lands, and areas subject to biotic and abiotic disturbances (e.g. recent burnings, roadsides, railways, trails, wastelands, gravel pits, abandoned and weeded areas) (Callihan, 1990; Evans and Gunnell, 1982; Roché, 1992; Zouhar, 2009). Commonly found in disturbed habitats, the species is fully capable of invading well-vegetated communities usually dominated by mountain juniper (*Juniperus scopulorum* Sarg.), big sagebrush (*Artemisia tridentata*), grasslands dominated by blue bunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á.Löve), and the invasive annual cheatgrass (*Bromus tectorum* L.) pastures, rangelands, lightly shaded and forested areas, and riparian areas (Callihan et al., 1984; Farah et al., 1988; Grantham, 2015; West and Farah, 1989). Habitats infested with *I. tinctoria* (e.g. rangelands, pastures and croplands, and disturbed areas) were surveyed throughout Idaho and lists of trees, shrubs, grasses, and perennial herbs species associated with each habitat were compiled (Callihan et al., 1984).

History

I. tinctoria has a long fragmented history of introductions across the northern hemisphere. Most records document its cultivation throughout Europe and its eventual introduction to and expansion across western North America. Carbonized seeds date its cultivation in Roissy, France to the fifth or fourth century BCE, and seeds and pod fragments date its civilization in Dragonby, England to the first century BCE and A.D. (Van der Veen et al., 1993; Zech-Matterne and Leconte, 2010). Records of its introduction into Italy, via the Romans, and its cultivation in Pompeii date prior to the eruption of Mount Vesuvius (Guarino et al., 2000). England (Somerset and Lincolnshire), France (Normandy, Somme, Languedoc), Germany (Jülich, Thuringia), and Italy (Piedmont, Tuscany) were the known woad districts and centers of production during the Middle Ages, starting around 1200 A.D. (Gilbert and Cooke, 2001; Oberthür et al., 2004a; Stoker et al., 1998a). Cultivation and production of *I. tinctoria* and its dye continued through the 17th century when the industry declined due to competition with imported indigo obtained from tropical *Indigofera* species (Fabaceae) (Kokubun et al., 1998). The industry collapsed in the late 19th and early 20th century with the production of synthetic indigo (Gilbert and Cooke, 2001). Commercial production ceased in England after 1932 (Gibson, n.d.).

I. tinctoria has been introduced at least three times into North America, (Corbett, 1973; King, 1967; Robbins et al., 1951). During the peak of indigo production in Europe, the textile crop was deliberately introduced into Virginia and adjacent states during the colonial period for cultivation (Corbett, 1973; DiTomaso and Kyser, 2013). When cultivation was abandoned, naturalized populations of *I. tinctoria* spread throughout parts of eastern North America (Corbett, 1973; Young and Evans, 1971). In the early 20th century, a contaminated alfalfa seed shipment from Ireland introduced *I. tinctoria* to the Marlahan Ranch in Scott Valley, Siskiyou County, northern California (Robbins et al., 1951; Young and Evans, 1977). Alternatively, the introduction into northern California may have been due to contaminated piano packing material (J. Aceves, pers. comm.). This introduction is recognized as the source for infestation throughout California, Oregon, and western Washington (Callihan, 1990). While around 1910, a separate introduction of contaminated alfalfa seed introduced *I. tinctoria* into Utah, near Brigham City (King, 1967; Reeves, 2010). The naturalized weed remained unnoticed until it was first reported in 1917 (Varga and Evans, 1978). *I. tinctoria*

was officially recognized in Utah upon identification in 1932 when specimens near the railway in Perry were collected for the Utah State University herbarium (Evans and Gunnell, 1982; Varga and Evans, 1978). Once established, the weed quickly spread through southeastern Idaho, northern Utah, western Wyoming, and parts of Montana (Callihan et al., 1984; Pokorny and Krueger-Mangold, 2007). Recent molecular work determined that there are two distinct *I. tinctoria* lineages found in the western United States, one in Northern California and southern Oregon, and another in the Great Basin (J. Gaskin, pers. comm.).

Growth and development

Morphology

The root system constitutes over 60% of the overall plant biomass after the first year of growth (Hakala et al., 2009). Lateral roots develop predominantly during the second year of growth in the upper 20 to 30 cm and branch up to 40 cm laterally, enabling *I. tinctoria* to utilize available soil resources well (Farah, 1987; Farah et al., 1988). The succulent and lightly pubescent leaves enhance water collection and storage, and the winged silicles are adapted to wind and other modes of dispersal (see Section 8) (Callihan, 1990; Callihan et al., 1984; Campeol et al., 2006; Farah et al., 1988; Hurry, 1930; Pokorny and Krueger-Mangold, 2007).

I. tinctoria demonstrates high phenotypic plasticity in response to varying light and soil water conditions, as well as seasonality across heterogeneous environments (see (c) Physiological data below). As opposed to strictly investing resources in plant elongation to shade out its competitors, to strengthen its competitive edge under shaded conditions, resources are equally allocated to different aboveground growth parameters (i.e. leaf area, specific leaf area, and root to shoot ratio) (Monaco et al., 2005). Overall plant performance and biomass production increase with soil moisture availability, but ample soil moisture is not a requirement for the drought-tolerant species (Campeol et al., 2006; Monaco et al., 2005). The suggested annual precipitation requirements for *I. tinctoria* range from 356 mm to 457 mm (Parker, 1975). Plants exhibit low plasticity in response to soil nitrogen (Monaco et al., 2005).

Perennation

In areas with ample water availability during the autumn months and moderate winters, which enables rapid growth (e.g. central Europe), *I. tinctoria* behaves like a winter annual (H. Hinz, pers. comm.). While during the dry and/or warmer autumn months (e.g. Mediterranean, or inland western North America), its life cycle type is predominantly biennial to facultative short-lived perennial (Callihan, 1990; Guarino et al., 2000; King, 1966; McConnell et al., 1999). At the natural and experimental field sites located on the western slopes of the Wellsville Mountains, in Box Elder County, Utah, 12% and 13% of plants, respectively, remained in the vegetative rosette stage during the second year of growth with the potential to reproduce in the third year or later (Farah et al., 1988). Typically, plants are monocarpic (i.e. die following reproduction); however, polycarpic perennials (i.e. reproducing more than once during their lifetime) have been observed under laboratory conditions (Asghari, 1993) and in the field (R. Gibson II, unpublished data). The percentage of polycarpic perennials within a population has not been assessed (Asghari, 1993).

Physiological data

Leaves are rich in nutrients, specifically potassium, despite their low biomass (Hakala et al., 2009), and contain a higher overall content of glucosinolates than seeds (Mohn and Hamburger, 2008). The roots of *I. tinctoria* store more than half of the plant's total nitrogen, phosphorus, and magnesium (Hakala et al., 2009), and they synthesize and store high quantities of glucosinolates (Elliott and Stowe, 1971a). N-S fertilization, jasmonic acid, artificial wounding, and insect attack all significantly enhance the glucobrassicin concentrations in leaves (Galletti et al., 1999; Galletti et al., 2006).

Secondary metabolites of *I. tinctoria* have been studied extensively (Berkeley, 1961; Danz et al., 2002a; Mohn et al., 2009). The two classes of secondary metabolites recognized in fresh woad leaves are indigo precursors (Beijerinck, 1900; Gilbert and Cooke, 2001; Schunk, 1855) and glucosinolates (Mohn et al., 2008; Van Dam et al., 2009).

Indigo precursors are glucosides that form varieties of indigo through hydrolysis and oxidation (i.e. the conversion of precursors into indoxyl groups and the spontaneous combination of two indoxyl groups when exposed to air) (Campeol et al., 2006; Hancock, 1997). The dominant indigo precursor found in *I. tinctoria* is isatan B (Epstein et al., 1967);

however, recent metabolite profiling and chemical restructuring states the composition is mainly isatan A followed by isatan B (Oberthür et al., 2004b). Additional indigo precursors include isatan C (though no definitive structure was proposed) and indican (Gilbert et al., 2000; Maugard et al., 2001; Strobel and Gröger, 1989). Different shades and colors of indigo dye are determined by the composition and concentration of indigo precursors and impurities (Hancock, 1997). The main factors influencing the production of indigo precursors, and subsequently indigo yield, are soil moisture and composition, plant and leaf age, seasonality, and landrace (i.e. species variants produced via cultivation) (Angelini et al., 2007; Bruni, 1858; De Lastreyrie, 1811; Kokubun et al., 1998; Rocha et al., 2011a; Turkmen et al., 2004).

Light intensity and light spectrum play a vital role in the production of indigo (Tozzi et al., 2005; Turkmen et al., 2004). Indigo production is greatest when leaves are harvested after three or four days of sunshine (greater than 1400 μmol m^{-2} s^{-1}) (Stoker et al., 1998b). The concentration of indigo precursors per leaf dry weight (specifically isatan B) are positively influenced by water stress (Campeol et al., 2006). Factors of plant biology that do not influence indigo production are plant size, temperature, and high nitrogen content (Sales et al., 2006; Stoker et al., 1998b). In an attempt to reintroduce *I. tinctoria* as a natural source of indigo dye for commercial production in Europe, studies seek to understand the biosynthetic pathways of indigo production (Clark et al., 1993; Maier et al., 1990; Schraudolf, 1968; Xia and Zenk, 1992), historic and alternative methods of extraction (Garcia-Macias and John, 2004; Kokubun et al., 1998; Oberthür et al., 2004a; Stoker et al., 1998a), and the agronomic aspects for the optimization of production (Angelini et al., 2007; Campeol et al., 2006; Spataro and Negri, 2008a; Stoker et al., 1998b; Tozzi et al., 2005).

Glucosinolate composition and concentration is organ specific in *I. tinctoria* (Mohn and Hamburger, 2008). In Switzerland, the composition and concentration of glucosinolates stored in seeds of Thüringer Waid was studied and found to contain mainly aliphatic glucosinolates: sinigrin, progoitin 90 mg, epiprogoitrin 130 mg, and gluconpain 55 mg/100 g dry weight (Fréchard et al., 2001; Mohn and Hamburger, 2008). Seeds of Thüringer Waid also contained glucotropaeolin (an aromatic glucosinolate) and indolic glucosinolates: glucobrassicin 45 mg, 4-hydroxyglucobrassicin 15 mg, and neoglucobrassicin 10 mg/100 g dry weight (Mohn and Hamburger, 2008); in contrast, only glucobrassicin was found in seeds during an initial study in Connecticut, 230 mg/100 g fresh weight (Elliott and Stowe, 1971a).

Unique to seeds are two epimeric mixtures found in French Woad: glucoisatisin and 3`-hydroxyglucoisatisin (Fréchard et al., 2001). Similar studies on Thüringer Waid only found glucoisatisin, 90 mg/100 g dry weight (Mohn and Hamburger, 2008). The silicles (seedpods) of *I. tinctoria* are coated with an unidentified water-soluble chemical solution that inhibits germination and root elongation (Young and Evans, 1971). The glucosinolate profile of silicles has not been assessed.

The glucosinolate profile of leaves is primarily indolic glucosinolates (Galletti et al., 1999) (i.e. sulfoglucobrassicin, which is unique to leaves and shoots (Elliott and Stowe, 1971a; Elliott and Stowe, 1971b), glucobrassicin; 4-hydroxyglucobrassicin; and neoglucobrassicin) and with lower concentrations of aliphatic glucosinolates: progoitrin, epiprogoitrin (0.1 – 1.3 μmol/g) and gluconapin (0.01 – 0.04 μmol/g) (Mohn et al., 2008). Sulfoglucobrassicin and glucobrassicin are found in concentrations approximately 100-fold higher than progoitrin and epiprogoitrin (Mohn et al., 2008).

Roots contain a mixture of aliphatic and indolic glucosinolates, but most research focuses on indolic glucosinolates (Galletti et al., 1999). Cultured in the medium for one week, excised roots contained glucobrassicin and neoglucobrassicin (9.8 mg and 16.8 mg/100 g fresh weight, respectively) (Elliott and Stowe, 1971a). Additionally, small quantities of glucobrassicin and neoglucobrassicin (0.15 and 0.38 mg/100 g, respectively) were released into the medium during the same study; however, the effect of the indolic glucosinolates on germination and root elongation of other plant species was not assessed (Elliott and Stowe, 1971a).

In Switzerland, the variation in glucosinolate concentration of five *I. tinctoria* landraces showed a similar pattern to seasonal changes. Assessed during the rosette stage, aliphatic glucosinolates peaked towards the end of June followed by a steady seasonal decline, with the exception of French Woad peaking earlier. Indolic glucosinolates were lowest in young plantlets during early spring and increased several-fold over the course of the season, peaking in July and August. A closer analysis revealed the overall increase in concentration of glucosinolates through the season was due to sulfoglucobrassicin, whereas glucobrassicin remained relatively constant, while 4-hydroxyglucobrassicin and neoglucobrassicin showed no consistent pattern and occurred at concentrations approximately 100-fold lower (Mohn et al., 2008). In contrast, rosettes in Connecticut showed the high

concentration of indolic glucosinolates in May, which subsequently decreased in June and plateaued through the summer months, before peaking in September, which coincides with leaf senescence (Elliott and Stowe, 1971a). Glucobrassicin, neoglucobrassicin and sulfoglucobrassicin all show similar patterns in seasonal variation (Elliott and Stowe, 1971a; Kutáček et al., 1959). Indolic glucosinolate concentrations of second year rosettes peaked in April and May and drastically decreased as plants bolted and flowered (Elliott and Stowe, 1971a). Etiolation affected the synthesis of glucosinates in leaves and shoots (Elliott and Stowe, 1971a).

Phenology

Seeds of *I. tinctoria* have no dormancy (Evans and Gunnell, 1982) and can germinate in the autumn months, following reproduction, or the following spring (Farah et al., 1988). Seeds are able to germinate in as little as 7 to 10 days (Hurry, 1930), or 4 to 7 days if the silicle is cracked or removed (Young and Evans, 1971). In the North American Intermountain West, 1% of plants flower in the first year (winter annuals) whereas 50% to 87% flower in the second year (biennials) (Farah et al., 1988). During the late summer months, high temperatures and drought may induce summer dormancy to prevent water loss, premature leaf senescence, and mortality (Farah et al., 1988). Growth resumes during the autumn months before rosettes enter winter dormancy (Hurry, 1930). Vernalization, i.e. temperatures below 4 °C for 23 to 47 days, is required to trigger the change from vegetative meristem to reproductive meristem (Asghari et al., 1992; Asghari, 1993). The change from vegetative to reproductive meristem requires a threshold rosette size by the end of the growing season. If a plant lacks sufficient below-ground reserves to sustain successful reproduction, the plant will remain in the rosette stage through the following growing season (Evans, 1991). Plants can persist as rosettes for multiple years (Jacobs and Pokorny, 2007). Growth resumes in young rosettes and above ground senesced plants less than a week after snowmelt (West and Farah, 1989). On the Wellsville Mountains in Box Elder County, Utah, mature rosettes bolt at a mean of 10 cm per week over a span of 8 weeks, from mid-April to the end of May, then flower, and set seed (Farah et al., 1988). Mature fruits are dispersed around the end of July as the pedicels and lignified stems break down (Farah et al., 1988).

Mycorrhiza

I. tinctoria is non-mycorrhizal (Pendleton and Smith, 1983).

Reproduction

Floral biology

I. tinctoria is considered a self-incompatible, obligate outbreeder (Spataro et al., 2007); however, a recent study found that the plant has some capability for self-compatibility (Spataro and Negri, 2008b). In the manipulative study, self-pollinated plants produced lighter and fewer silicles than outcrossed plants (7.1 g to 44.1 g and 6.0 mg to 8.0 mg, respectively), while the weight and quantity of silicles produced by controlled-outcrossed plants was indifferent to those of self-pollinated plants (11.9 g and 6.0 mg, respectively). Nevertheless, the silicles produced by self-pollinated plants were 82% less viable than the silicles of outcrossed plants (Spataro and Negri, 2008b). In the American Intermountain West, the main form of pollination is unknown. No information on insect pollinators (specialists or generalists) was found. Research on pollination and pollinators in the introduced range will be important to determine whether 1) the plant is providing services to native generalist or specialist insect species and vice versa, and 2) *I. tinctoria* is predominately pollinated by insects or wind.

Seed production and dispersal

The reproductive output of *I. tinctoria* is dependent on plant age and size, and the region and habitat in which the plant grows (Farah et al., 1988). *I. tinctoria* produces 300 to 600 seeds per plant in central Europe (H. Hinz, pers. comm.) and parts of the North American Intermountain West (Farah et al., 1988). However, plants have been recorded to produce an average of 2,800 to 3,400 seeds per plant in the Mediterranean region (Rocha et al., 2011a), while the highest number of seeds recorded was more than 10,000 seeds per plant (McConnell et al., 1999). In natural populations in the American Intermountain West, seed production varies from less than 50 to more than 5,000 seeds per flowering stem (R. Gibson II, unpublished data). The number of reproductive stems per plant varies between 1 and 3, but can exceed over 10 stems per plant under optimal conditions (R. Gibson II, unpublished data).

Silicles require an abrasive force (wind or rain) to be detached from the lignified stem (Farah et al., 1988). Although wind has been recognized as a primary dispersal mechanism of *I. tinctoria* (Hurry, 1930), it is not the sole mechanism for long-distance dispersal. A study on the western slopes of the Wellsville Mountains in Box Elder County, Utah, recorded that 95% of silicles fell within a 54 cm radius of the mother plant, with a maximum distance of 2.4 m (Farah et al., 1988). Wind and gravity may increase seed dispersal from mother plants found on agricultural terraces and rangeland slopes (Farah, 1987). Additionally, silicles can remain attached to the mother plant past the first snowfall and be blown across the frozen snowpack (Farah et al., 1988). Other documented plausible means of long distance dispersal include silicles floating downstream, being carried by birds or ants, and epizoochory on mammals (Callihan et al., 1984; Farah, 1987; Farah et al., 1988).

Humans also facilitate long-distance seed dispersal of *I. tinctoria*. In farming communities, silicles are spread via hay bales, seed crop, bedding, and farm equipment (Callihan, 1990; NWCB, 1999). In disturbed areas and high traffic transportation corridors, silicles can attach to clothing and footwear by means of the pedicel, which acts as a hook (Farah et al., 1988). Furthermore, silicles can adhere to vehicle tires or get caught in undercarriages via mud or debris (Callihan, 1990; Farah et al., 1988). Other human influenced vectors of dispersal include trains and construction equipment (Pokorny and Krueger-Mangold, 2007).

Seed banks, seed viability and germination

Little is known about the seed bank of *I. tinctoria*, and seed viability and germination have not been assessed beyond an 18-month period (Farah et al., 1988). Seed viability is generally thought to be short-lived (Farah et al., 1988); however, indehiscent silicles buried in the soil (seed bank) can remain viable for over 10 years (Hurry, 1930; King, 1966; Jacobs and Pokorny, 2007). On the western slopes of the Wellsville Mountains in Box Elder County, Utah, silicles buried 1 cm below the surface showed no difference in seed viability during the 10 month study period, while the germination of threshed seeds decreased from 99% in September to 44% the following May (Farah et al., 1988). The combination of seed viability and germination varied between 73% and 100% throughout the study period, with the highest percentages occurring during the months of September, March, April, and May (Farah et al.,

1988). No seeds germinated after 18 months suggesting that seeds 1) have limited viability on the soil surface, 2) are triggered into dormancy with time, or 3) experience significant predation and/or pathogen attack (Farah et al., 1988). Germination success in the field was independent of precipitation or mean monthly temperature (Farah et al., 1988).

In laboratory experiments, seeds of both threshed and indehiscent silicles germinated when incubated at temperatures ranging from 3 °C to 25 °C (Young and Evans, 1971). Seeds of indehiscent silicles had a reduced percentage of germination success and seedling elongation to those of threshed silicles, 76% to 100% and 50%, respectively (Young and Evans, 1971). The silicle is recognized as a protective barrier and can induce dormancy (Jacobs and Pokorny, 2007; Young and Evans, 1971). Each silicle is coated with an allelopathic substance, which was found to reduce the germination success of *I. tinctoria* and other plant species (Young and Evans, 1971). The germination-inhibiting substance is water-soluble and leachable (Evans and Gunnell, 1982; Young and Evans, 1976). The leaching of the allelopathic substance from the silicles and soil is suspected to be a partial cause for the high spring germination rate (70% to 80%) (Farah et al., 1988; Young and Evans, 1976). Additional factors that decrease seed viability and germination are self-fertilization (82% reduction) (Spataro and Negri, 2008b) and shading (85% reduction) (Tozzi et al., 2005)

Vegetative reproduction

I. tinctoria can reproduce vegetatively in the form of adventitious buds on the root crown and upper roots (Asghari, 1993; Callihan, 1990; Evans, 1991; Zouhar, 2009). Mature plants can vegetatively reproduce one to three times during a reproductive cycle before senescing; this process is dependent on the frequency and the percentage of the above ground biomass removed (see Section 13) (West and Farah, 1989). The impact of resource availability on vegetative reproduction has not been assessed.

Hybrids

There are no reports of interspecific hybridization involving *I. tinctoria*.

Population Dynamics

The population dynamics of *I. tinctoria* was studied at two natural sites and one artificially established site on the western slopes of the Wellsville Mountains in Box Elder County, Utah (Farah et al., 1988). Across the two natural sites in spring 1982, 180 young rosettes were marked, of which 65% died the first growing season and only 1% flowered. In spring 1983, approximately half of the surviving plants flowered and fruited. At the beginning of September 1984, 100,000 seeds were sown at an experimental site. By October, 285 young rosettes established (0.3%), the survivors of which overwintered twice before reproducing. In spring 1985, an additional 2,664 young rosettes established (2.7%), none of which flowered the same year. The following spring, 87% of the surviving plants from both germination seasons flowered and fruited. No herbivory or pathogen attack was found at either site (Farah et al., 1988).

At the experimental site, young rosettes had the highest risk of mortality (77%), in contrast to mature rosettes, which had a lower probability of mortality (19%) (Farah et al., 1988). The rate of mortality was independent of the plant's germination season. Throughout most of the study, autumn-germinated plants had slightly larger rosettes and taller stems than spring-germinated plants (Farah et al., 1988). Additionally, autumn-germinated plants produced more fruits per plant than spring-germinated plants (563 vs. 345, respectively). Regardless of the reproductive advantages of autumn-germinated plants (Sales et al., 2006), spring-germinated plants contribute more to the overall population growth due to higher germination rates (Farah et al., 1988). While this study provided an overview of the biology, phenology, and population biology of *I. tinctoria,* demographic studies on the population dynamics and analysis of key mortality factors are still needed for this invasive plant species (Farah et al., 1988).

Response to herbicides and other chemicals

For the control of *I. tinctoria*, herbicides are most commonly and preferentially used. Herbicides are best utilized in agricultural settings, combined with the competition by the respective crop, and along roadsides (Varga and Evans, 1978; Kropp and Darrow, 2006). The most commonly used herbicide chemicals and mixtures include: 2,4-D; chlorsulfuron; imazapic; metsulfuron; 2,4-D combined with amitrol, chlorsulfuron, or metsulfuron; and

chlorsulfuon combined with aminocyclopyrachlor (Callihan, 1990; DiTomaso and Kyser, 2013; Evans and Gunnell, 1982; Jacobs and Pokorny, 2007; PNW, 2016; USDA Forest Service, 2014; Varga and Evans, 1978). However, chlorsulfuon combined with aminocyclopyrachlor is not approved for use in California and in some Colorado counties due to the strong effects on non-target species (DiTomaso and Kyser, 2013; PNW, 2016). *I. tinctoria* is fairly tolerant to metribuzin, dicamba, and picloram, even under higher herbicide concentrations (Evans and Gunnell, 1982; Varga and Evans, 1978). The optimal time to treat infestations in the northwestern United States is in early spring, during the seedling and young rosette stages (Callihan, 1990); however, *I. tinctoria* remains susceptible to herbicidal treatment through the bolting stage (Varga and Evans, 1978).

Herbicide management of *I. tinctoria* infestations on federal lands, in rangelands, forests, and other inaccessible terrains is considered uneconomical and unfeasible in part due to 1) site remoteness, 2) damage to the land's overall productivity, 3) the low economic value of the lands, and 4) potential indirect effects on pollinators and pollinator services (Kropp et al., 1995; Obama, 2014; Varga and Evans, 1978). In addition, there are concerns about the potential of non-target effects of herbicides on native plant communities, which can indirectly aid the re-establishment of *I. tinctoria* from the soil seed bank (Kropp et al., 1995).

Response to other human manipulation

For the management of *I. tinctoria* infestations, the two forms of human manipulation include mechanical and manual. Mechanical manipulation (i.e. tilling, mowing/clipping, and various means of cultivation) is commonly restricted to agricultural ecosystems due to equipment availability and accessibility (Callihan, 1990; Evans and Gunnell, 1982; Varga and Evans, 1978). Mechanical manipulation in pastures and rangelands is considered ineffective. Mowing or clipping plants usually requires multiple treatments to reduce seed production or achieve plant mortality because the removal of the reproductive stem(s) can 1) activate bud production on the root crown and upper roots, or 2) delay flowering to the following year (West and Farah, 1989; DiTomaso and Kyser, 2013). However, on the western slopes of the Wellsville Mountains in Box Elder County, Utah, a single clip that removed 90% of the reproductive stem at or after peak flowering reduced the plants reproductive output by 77% and significantly increased mortality (Fuller, 1985; West and Farah, 1989). To ensure the

mortality of *I. tinctoria*, the plant must be either clipped below the root crown (5 cm below the soil surface) (Callihan, 1990; Fuller, 1985), or clipped and then spot treated with herbicide (Pokorny and Krueger-Mangold, 2007).

Manual manipulation (i.e. hand-pulling, hoeing, digging, and planting adaptive perennial grasses) is beneficial during any growth stage. Hand pulling and digging, specifically, are recognized as effective methods of controlling and even eradicating *I. tinctoria* on smaller scales and have been used to reduce infestations as large as 25 ha (Callihan, 1990; Dorst et al., 1994; Jacobs and Pokorny, 2007; Pokorny and Krueger-Mangold, 2007; Varga and Evans, 1978; Young and Evans, 1977). Over 14 summers, from 1980 to 1993, volunteers exceeded 3,175 hours of manual manipulation of *I. tinctoria* infestations along the foothill rangelands near Logan, Utah (Dorst et al., 1994). The average amount of hours required to manage each site fell 49% from the first to the second year. Within 8 years, the average amount of hours spent per site was reduced by 90% from the first year and *I. tinctoria* populations declined by at least 95% (Dorst et al., 1994). Similarly, the Montana Cooperative Dyer's Woad Project was formed in 1984 and has eradicated *I. tinctoria* from 9 of the 13 known infested Montanan counties as of 2005 (Pokorny and Krueger-Mangold, 2007). In the counties where *I. tinctoria* persists, the proportion of plants that remain is low (1-5% of the current average canopy) (Pokorny and Krueger-Mangold, 2007). The cost of the project between 1985 and 2005 was estimated at $225,000 with an average annual cost of $20,286 between 1999 and 2005. It was estimated that if populations in all 13 counties were left unattended until 2005, management costs would have been close to $2 million for one year of management alone (Pokorny and Krueger-Mangold, 2007). Continuous monitoring and management is required to ensure *I. tinctoria* is not reintroduced from adjacent heavily infested lands. To accomplish this, the Montana Cooperative Dyer's Woad Project offers a $50 incentive for every new infestation reported that is located more than 0.8 km from a known population (Enz and Pokorny, 2005; Pokorny and Krueger-Mangold, 2007). To ensure the control of *I. tinctoria*, it is advised to bag flowering and seeding plants to prevent the deposit and dispersal of seeds (Dorst et al., 1994; Pokorny and Krueger-Mangold, 2007).

Prescribed burns are not an encouraged method of control. The impact of fire on *I. tinctoria* is poorly understood (DiTomaso and Kyser, 2013). Fire can remove the above-

ground biomass but leave the root system intact, which only delays the plant's reproduction until the following year (DiTomaso and Kyser, 2013; Zouhar, 2009). Also, as an early successional species, seeds buried in the soil will likely survive a fire and reestablish across the burned area at a greater rate the following year (Zouhar, 2009).

Response to herbivory, disease, and high plant parasites

Herbivory

Mammals

In Europe, sheep graze on *I. tinctoria* remnants only after the first or second frost, due to the sweetish taste (Hurry, 1930). In the North American Intermountain West, the common grazing period is early spring. After the release of 150 sheep into an *I. tinctoria* infested pasture, only 48 of the 300 tagged plants showed signs of grazing, of which only an average of 39% of the above ground biomass was removed (West and Farah, 1989). Grazing showed no significant impact on the percentage of plants flowering, the average silicle production and weight, or plant mortality (West and Farah, 1989). Although *I. tinctoria* is considered unpalatable to livestock, especially after plants begin to produce reproductive stems, it is not known to be toxic (DiTomaso and Kyser, 2013; Hansen and Bloem, 2006; Young, 1988). Livestock and deer appear to prefer native foliage, and will avoid *I. tinctoria* rosettes even when native vegetation is unavailable (Kropp et al., 1996; West and Farah, 1989).

Birds and other vertebrates

No reports or studies of herbivory by birds or other vertebrates were found.

Insects

There was no information readily available on herbivorous or pollinating insects utilizing *I. tinctoria* in North America. However, studies and anecdotal records were collected on infestations in Cache Valley, Utah (Evans and Gunnell, 1982).

Insects recorded specifically on *I. tinctoria* agricultural cultivars in Europe include the cabbage aphid (*Aphis brassicae* L.), the turnip fly (*Phyllotreta nemorun* L.), the cabbage caterpillar (*Pieris brassicae* L.), cabbage webworm (*Hellula undalis* F.), and certain *Acrididae* species (Hurry, 1930; Sales et al., 2006).

Between 2004 and 2006, CABI in Switzerland surveyed a total of 40 field sites in Europe and parts of eastern and central Asia for herbivorous insects feeding on *I. tinctoria* (Hinz et al., 2007; Hinz et al., 2005). Although bolting or flowering plants generally had higher attack rates than rosettes, the attack rates of herbivorous insects ranged between 50% and 100% of plants in the field. Reproductive stems heavily attacked by herbivores were stunted or completely dead. External feeders included lepidopteran or leaf beetle larvae, while internal mining of stems and roots predominantly included flea beetles and weevils, but also cerambycid larvae (Hinz et al., 2005). Of the 52 insect species that were collected from *I. tinctoria*, 33 species are known to feed or develop strictly on *Brassicaceae* plants (Hinz et al., 2007). In addition, searches through the European literature, led to a total of 62 herbivore insect species associated with *I. tinctoria* (Hinz et al., 2007).

Based on those surveys, nine candidate herbivore species were prioritized for the biological control of *I. tinctoria*: two root mining weevils, *Aulacobaris fallax* Brisout and *A. licens* Reitter; the root crown weevil, *Ceutorhynchus rusticus* Gyllenhal; two stem mining flea beetles, *Psylliodes isatidis* Heikertinger and *P. tricolor* Wiese; an undescribed stem mining *Lixus* species; the flower feeding beetle, *Meligethes anthracinus* Brisout; and two seed feeding weevils, *C. peyerimhoffi* Hustache and *Bruchela exigua* Motschulsky (= *Urodon exiguus*) (Cortat et al., 2008; Gerber et al., 2009; Hinz et al., 2008; Hinz et al., 2007). Of these candidate species, four insects: *A. fallax*, *P. isatidis*, *C. rusticus*, and *C. peyerimhoffi* were studied in greater detail.

A. fallax was assessed in no-choice and multiple-choice host-specificity tests and attacked 16 of 39 and 7 of 17 test plants species, respectively, most or all of which were native to North America (Gerber et al., 2009). These data suggested that *A. fallax* is not sufficiently host-specific on *I. tinctoria* and investigations on this candidate species were suspended (Hinz et al., 2010; Hinz et al., 2011; Hinz et al., 2012; Hinz et al., 2013). *P. isatidis* demonstrated a relatively narrow host range when conducted in multiple-choice host-specificity tests and under natural conditions (Hinz et al., 2010; Hinz et al., 2011; Hinz et al., 2012; Hinz et al., 2013). Unfortunately, one adult *P. isatidis* emerged from a *B. rapa* L. plant, (white turnip), an economically important crop. Furthermore, the recorded physiological larval host range includes two additional economic *Brassica* crops (*B. juncea* (L.) Czern. and *B. napus* L.) and a threatened and endangered North American species (*Boechera hoffmannii*

(Munz) Al-Shehbaz) (Hinz et al., 2014). Based on these data, further research involving *P. isatidis* was terminated in 2013.

Extensive host-specificity tests were conducted with the root-crown feeding weevil, *C. rusticus*, between 2005 and 2015. Of over 100 test plant species exposed under no choice conditions, *C. rusticus* was able to develop to adult on seven species in six genera, six species of which are native to North America (H. Hinz, pers. comm.). However, when these test plants were assessed under open field conditions together with *I. tinctoria*, oviposition was only occasionally recorded on individual test plants (Hinz et al., 2014). In addition, *C. rusticus* has shown to significantly reduce the vigor, survival, and seed output of *I. tinctoria* (H. Hinz and E. Gerber, unpublished data). No petition has currently been submitted to regulatory authorities for the field release of *C. rusticus* into North America (H. Hinz, pers. comm.). Additional experiments aimed to demonstrate the realized host range of *C. rusticus* is sufficiently narrow and suitable for introduction to North America are currently underway.

Thus far, the seed feeding weevil, *C. peyerimhoffi*, has been the most promising biological control candidate tested. After no-choice, single-choice, and multiple-choice oviposition and larval development host-specificity tests, *C. peyerimhoffi* is concluded to have an exceptionally narrow host range (Hinz et al., 2010; Hinz et al., 2011; Hinz et al., 2012; Hinz et al., 2013). A petition for field release of *C. peyerimhoffi* into North America may be prepared in 2017/18 (Hinz et al., 2014). To date, no classical biological control candidates have been released in North America (Callihan and Miller, n.d.; Hinz et al., 2014; Winston et al., 2014).

Nematodes and other invertebrates

In Europe, four nematode species were recorded on *I. tinctoria*: *Aphelenchoides ritzemabosi* (Schwartz) Steiner & Buhrer, *Ditylenchus dipsaci* Kühn, *Heterodera schachtii* Schmidt, and *Meloidogyne* spp. (Buhr, 1964; Goodey et al., 1965; Sales et al., 2006). *A. ritzemabosi* is a pathogenic nematode, endoparasitic to leaves and ectoparasitic to buds and root tissue (Goodey et al., 1965). The latter three nematode species are gall formers that feed on the stems and petioles, or strictly the root tissues. Other invertebrates recorded on *I. tinctoria* in Europe include snails (Hurry, 1930). No information was found for nematodes or other invertebrates on *I. tinctoria* in Asia or North America.

Diseases

Fungi

In Europe, eight fungi species were recorded on *I. tinctoria*: *Aecidium isatidis* (F.) Re., *Ophiobolus herbarum* (G. H. Otth.) Sacc., *Cicinnobolus cesatii* de Bary, *Monilia glasti* Plowr., *Oidium erysiphoides* Fr., *Septoria isatidis* Savul & Sandu, *Erysiphe cruciferarum* Opiz ex L. Junell, and *Puccinia trabutii* Roum & Sacc. (Gaumann, 1959; Hurry, 1930). All the aforementioned species have multiple hosts with the exception of *S. isatidis*. No information was found on these fungi species with regard to the biological control potential for *I. tinctoria*.

In May of 1978, a rust fungus was discovered on *I. tinctoria* in the isolated North American foothills near Grace, Idaho, and tentatively identified as *Puccinia thlaspeos* C. Schub (Daines, 1988; Lovic et al., 1988). *P. thlaspeos* is a polyphyletic species, and a member of the order Uredinales in the family Pucciniaceae, which includes the two largest rust genera: *Uromyces* and *Puccinia* (Cummins et al., 2003; Kropp et al., 1997). The genus *Puccinia* consists of highly specialized biotrophs, i.e. host specific species that require living tissue to grow and reproduce. *P. thlaspeos* infects various members in the family *Brassicaceae* in North America (Colorado, Idaho, and Utah), particularly species in the genera *Arabis* and *Thlaspi* (Arthur and Cummins, 1962; Farr and Rossman; Lovic et al., 1988).

The host specificity of *P. thlaspeos* strains vary with regard to different *Brassicaceae* host plants (Kropp et al., 1995). As its first reported occurrence on *I. tinctoria*, the identification of the rust was questioned when *P. thlaspeos* was found only to infect *I. tinctoria* (Kropp et al., 1997). Known host plants of *P. thlaspeos* present in close proximity to infected *I. tinctoria* remained uninfected (Kropp et al., 1995). In addition, morphological differences were found between the two strains of *P. thlaspeos* (Kropp et al., 1997). Additionally, subsequent host-specificity tests indicated that the rust strain discovered on *I. tinctoria* might not be *P. thlaspeos* (Environmental Protection Agency, 2002; Kropp et al., 1997; Mandelbaum and Kough, 2002). Alternatively, morphological characteristics, DNA sequence analyses, and the regional infestations suggested the rust is closely related to *P. consimilis* (a native, autoecious, systemic rust only found in the Rocky Mountain Range) and that it may actually be derived from a strain of *P. consimilis* that shifted from a native host

plant onto *I. tinctoria* (Environmental Protection Agency, 2002; Kropp et al., 1997). Based on these data, the strain of *P. thlaspeos* discovered on *I. tinctoria* is considered to be a member of the *P. monoica* rust complex and indigenous to the Intermountain range of North America (Kropp et al., 1997). It was classified as *P. thlaspeos* 'woad strain' and is commonly referred to as 'dyer's woad rust'; however, the true identification of the rust remains unknown (Environmental Protection Agency, 2002; Mandelbaum and Kough, 2002).

P. thlaspeos 'woad strain' was originally discovered in southeastern Idaho (Lovic et al., 1988). Over 20 years, its distribution slowly expanded roughly 300 km, into western Wyoming and throughout northern and central Utah, as far south as Salt Lake City (Daines, 1988; Kropp et al., 2002). By 1997, rust-free populations of *I. tinctoria* were difficult to locate within the area (Flint and Thomson, 2000). In City Creek Canyon, near Salt Lake City, the rate of rust dispersal averaged 14.6 m and 10.0 m by spring 1998 and 1999, respectively (Kropp et al., 2002). Long-distance dispersal events are rare and assumed to only occur when environmental conditions are ideal and/or facilitated by human assistance. However, once *P. thlaspeos* 'woad strain' has established in an infestation, it is anticipated to spread on its own (Kropp et al., 2002). The ultimate distribution of the rust is dependent on the range of *I. tinctoria*. Environmental conditions suitable for the development of the *P. thlaspeos* 'woad strain' have been unexplored outside its current distribution.

P. thlaspeos 'woad strain' is a systemic and autoecious rust fungus. It completes its life cycle on *I. tinctoria* in three stages: the spermogonial, telial, and basidial (Lovic et al., 1988). The spermogonial stage consists of bright yellow spermatia commonly found on new growth, specifically on the underside of leaves (hypophyllous) (Flint and Thomson, 2000). As the leaves mature, spermatia are intermingled with prominent and distinct light-brown or cinnamon-colored teliosori. The teliosori change color to ash-gray after the release of four basidiospores, which coincides with leaf senescence. Basidiospores are approximately 10 µm in length and 5 µm in width and height (Kropp et al., 1999).

Basidiospores are actively released and wind dispersed. When a *P. thlaspeos* 'woad strain' basidiospore lands on *I. tinctoria,* it adheres firmly to the leaf surface. Upon adherence, the basidiospore germinates and then produces an unbranched germ tube that is 2 µm to more than 30 µm in length (Kropp et al., 1999). *P. thlaspeos* 'woad strain' does not penetrate the stomata. Rust germ tubes form appressoria on the surface of epidermal cells

with symptoms of enzymatic disturbance of the epidermal wax layer from the germling (Kropp et al., 1999). The host plant is termed infected once the spore cytoplasm has entered the germ tube. After penetration of the host, *P. thlaspeos* 'woad strain' grows intercellular hyphae that penetrate leaf mesophyll and contiguous petiole parenchyma cells to form haustoria (Kropp et al., 1999). A haustorium establishes a parasitic connection with live plant cells. Hyphae spread through the leaf mesophyll and petiole parenchyma cells to the root system at an average rate of 0.25 cm per week (Kropp et al., 1996; Kropp et al., 1999). Host infection and colonization is asymptomatic for three to nine months as a result to pathogenesis-related gene suppression (i.e. PR-1, β-1, 3-glucanase and *ChiA*) and hyphae avoiding vascular tissue (Kropp et al., 1999; Thomas and Kropp, 2009; Thomas and Kropp, 2011).

I. tinctoria remains asymptomatic 9 to 45 weeks after infection (Flint et al., 1993; Flint and Thomson, 2000; Kropp et al., 1995). Symptoms of rust infection upon rosettes include chlorosis, leaf distortion, and stunted growth, followed by the production of spermogonia and telia on the underside of leaves (Kropp et al., 1995). When infected plants bolt, reproductive stems remain vegetative with most leaves distorted and chlorotic, and covered with spermogonia (Flint and Thomson, 2000). Upon maturation, reproductive structures are typically replaced with teliosori-infested leaves wrapped in a flower-like cluster, which often results in plant sterilization. However, in the instance reproductive structures remain, teliosori-infested flowers and silicles do not compromise seed viability but merely aid *P. thlaspeos* 'woad strain' reproduction (Kropp et al., 1996; Kropp et al., 2002) (Flint and Thomson, 2000). Additionally, *P. thlaspeos* 'woad strain' is not vertically transmitted to the next generation (Kropp et al., 2002).

The morphological changes and reduced seed production of *I. tinctoria* infected with *P. thlaspeos* 'woad strain' are likely induced by the alterations in plant hormones such as auxin-like and cytokinin-like compounds (Stirk et al., 2006). Extractions from rust infected *I. tinctoria* indicated *P. thlaspeos* 'woad strain' increases concentrations of auxin-like compounds and reduces concentrations of cytokinin-like compounds in rosettes (Stirk et al., 2006). The chlorotic leaves of rosettes and bolting plants are suspected to be the result of reduced chlorophyll concentrations caused by reduced concentrations of cytokinin-like compounds (Stirk et al., 2006). In bolting and flowering plants, *P. thlaspeos* 'woad strain'

significantly reduces concentrations of auxin-like compounds and slowly increases concentrations of cytokinin-like compounds, which prevents normal leaf senescence and stem lignification. The mechanisms responsible for the manipulation of plant hormone concentrations and their timing by *P. thlaspeos* 'woad strain' are unidentified (Stirk et al., 2006).

In 2002, *P. thlaspeos* 'woad strain' was federally approved in the United States as an augmentative biological control organism for the management of *I. tinctoria* (Environmental Protection Agency, 2002; Mandelbaum and Kough, 2002). Approved as a mycoherbicide known as the 'woad warrior,' *P. thlaspeos* 'woad strain' inoculum can be produced via traditional farming equipment and methods, and yields 80% of the number of basidiospores produced from inoculum harvested by hand in natural populations (Thomson and Kropp, 2004). Nevertheless, commercialization efforts failed due to 1) *P. thlaspeos* 'woad strain' already found in most infestations of *I. tinctoria* in the Intermountain West, and 2) the lack of a commercial backer (Flint and Thomson, 2000; Winston et al., 2014). The EPA permit for the 'woad warrior' in Utah expired in 2012 and was never renewed (Winston et al., 2014); however, the rust was recently approved by federal and state agencies for experimental release on *I. tinctoria* infestations in northern California. As of yet, the establishment of *P. thlaspeos* 'woad strain' in northern California has not been confirmed (Mark Schwärzlander, pers. comm.).

The natural incidence of *P. thlaspeos* 'woad strain' in *I. tinctoria* infestations is highly variable and appears to rest at relatively low levels, 1% to 36% (Daines, 1988; Flint and Thomson, 2000; Kropp et al., 2002; Lovic et al., 1988). The impact of *P. thlaspeos* 'woad strain' on *I. tinctoria* directly correlates with the percentage of plants infected within a population (R. Gibson II, unpublished data). Artificial inoculation can increase rust incidence and lead to reductions in plant density in subsequent years if inoculations are repeated annually (Kropp et al., 2002). Inoculation is achieved through the application of infected leaf material either directly on the plants or the adjacent soil (Kropp et al., 2002). Although infection of *I. tinctoria* can result from a single basidiospore, rust incidence increases with greater inoculum dosages (470 mg per plant was the maximum utilized in this study) (Kropp et al., 2002). Optimal conditions for natural infection and artificial inoculation typically occur in April and May, under warm and relatively humid weather conditions (between 10 ºC and

20 ºC, optimally 15 ºC) or during (Flint et al., 1993; Flint and Thomson, 2000; Kropp et al., 1996; Kropp et al., 1999). Inoculation applications include manual distribution by hand, or a hydro-seeder containing a mixture of either inoculum and hydromulch or inoculum and herbicide (Kropp et al., 2002; Kropp and Darrow, 2006). The application of *P. thlaspeos* 'woad strain' inoculum and herbicide is effective in reducing *I. tinctoria* plant densities in accessible infestations while, simultaneously, increasing rust incidence in said infestations with the anticipation that the rust will disperse to inaccessible infestations (Kropp and Darrow, 2006). *P. thlaspeos* 'woad strain' inoculum can be combined with either chlorsulfuron or metsulfuron herbicides, and the surfactant, Regulaid. Mycoherbicide application has no negative effects on teliospre viability or the dispersal of previously infected *I. tinctoria*, as long as spraying occurs after teliosori appear (Kropp and Darrow, 2006).

Bacteria

No information was located.

Viruses

No information was located.

Higher plant parasites

In Europe, two plant pathogens were recorded on *I. tinctoria*: *Plasmodiophora brassicae* Woron. and *Albugo candida* (Pers.) Ktze. (Buhr, 1964). Both plant pathogens infect multiple hosts in the family Brassicaceae.

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Chapter 2: The epidemiology and effect of the native rust fungus *Puccinia thlaspeos* on the invasive forb *Isatis tinctoria* (dyer's woad) in the Intermountain West

Introduction

The number of natural enemies that attack and parasitize invasive exotic plant species in their introduced range, and the intensity of their impact, are generally reduced when compared to the native range (Blossey, 2011; Colautti et al., 2004; Hinz and Schwarzländer, 2004; Mitchell and Power, 2003; Torchin et al., 2003; Torchin and Mitchell, 2004). Theory predicts (Keane and Crawley, 2002) and comparative studies confirm that exotic plant species are often in a state of release from their specialist natural enemies in the introduced range (Blair and Wolfe, 2004; Cripps et al., 2006; Liu and Stiling, 2006). Despite the escape from natural enemies upon introduction, exotic plant species accumulate herbivores and pathogens native to the introduced range over time through the occurrence of host range expansion, and/or host shifts (switching to a taxonomically related host) or host jumps (switching to an unrelated but ecologically associated host) (Agrawal and Kotanen, 2003; Mitchell and Power, 2003; Roy, 2001; van Klinken and Edwards, 2002).

Host shifts and jumps are recognized as common occurrences for fungal pathogens (Roy, 2001; Wingfield, 2003) and the main speciation events that drove the evolution and diversification of rust fungi (McTaggart et al., 2016; van der Merwe et al., 2008). For example, crucifer rusts within the *Puccinia monoica* complex have shown patterns of host jumping throughout the western United States (Roy, 2001). With the increase in globalization and agrotechnology, host shifts and jumps have become more common (Anderson et al., 2004; Coutinho et al., 1998; Slippers et al., 2005) along with novel ecosystems, i.e. new species associations and abundances within a given biome (Hobbs et al., 2006).

In relation to exotic plant species, most studies of novel associations primarily focus on insect herbivores (Blossey and Notzold, 1995; Hinz and Schwarzländer, 2004; Hokkanen and Pimentel, 1989; Maron and Vilà, 2001; Novotny et al., 2003; Olckers and Hulley, 1991; Tuda et al., 2009; Williams and Norton, 2012), while relatively little is known about novel plant-pathosystems (Blossey, 2011). Here we aim to evaluate the occurrence and effects of a

native North American rust fungus, *Puccinia thlaspeos* C. Schub., on the reproductive output of the invasive exotic Eurasian forb, *Isatis tinctoria* L.

Isatis tinctoria (dyer's woad) is a widely distributed, herbaceous forb in the Brassicaceae family (King, 1966). It is assumed native to the steppes and desert regions of the Caucasus Mountains to eastern Siberia and the Near East (Dunn, 1905; Evans and Gunnell, 1982; Jacobs and Pokorny, 2007). Cultivated for the production of blue dye, *I. tinctoria* has spread and naturalized throughout the northern hemisphere (most of Asia, Europe, and parts of North Africa) (Hurry, 1930; King, 1966; Rocha et al., 2011). The plant was first introduced to eastern North America deliberately during the 17th century, for the production of blue dye, where it naturalized (Corbett, 1973). Later, *I. tinctoria* was accidently introduced to western North America (California and Utah) during the early 20th century via contaminated alfalfa seed shipments from Ireland (King, 1967; Robbins et al., 1951; Young and Evans, 1977). It is a highly plastic species (Monaco et al., 2005) and capable of growing in diverse habitats, from disturbed roadsides, gravel pits, and wastelands to pastures, rangelands, well-vegetated grasslands, and lightly forested areas (Callihan et al., 1984; Grantham, 2015). The weed outcompetes native and desired vegetation (FWS, 2006; FWS, 2011) through its rapid growth (Jacobs and Pokorny, 2007), extensive two-layered rooting pattern (taproot and lateral roots) (Farah et al., 1988), and allelopathic properties (Young and Evans, 1971). Since its accidental introduction, *I. tinctoria* has spread vastly across the western United States and Canada, and been declared a noxious weed in 11 western states (Hansen and Bloem, 2006; USDA, 2010).

Almost 70 years after its establishment in the Intermountain West, in 1978, a systemic rust fungus identified as *Puccinia thlaspeos* was first discovered on *I. tinctoria* in Caribou County near Grace, Idaho (Callihan et al., 1984; Daines, 1988). *P. thlaspeos* is known to infect various members of the Brassicaceae family distributed throughout Europe and parts of North America (Colorado, Idaho, and Utah), most commonly *Arabis and Thlaspi* (Arthur and Cummins, 1962; Farr and Rossman; Lovic et al., 1988). The identification of the rust fungus was questioned when it was recorded to infect only *I. tinctoria*, even in close proximity of known hosts (Kropp et al., 1995). Host specificity tests indicated that the strain of rust fungus found on *I. tinctoria* is not *P. thlaspeos* (Environmental Protection Agency, 2002; Kropp et al., 1997; Mandelbaum and Kough, 2002). Morphological characteristics, DNA sequence

analyses, and the region of discovery provided further support towards this hypothesis and suggested the strain of rust fungus is either a close relative or derived from a strain of *P. consimilis* (a native, autoecious, systemic rust found only in the Rocky Mountains of western North America) that switched host plants onto *I. tinctoria* (Environmental Protection Agency, 2002; Kropp et al., 1997). This strain of rust fungus is considered a member of the *P. monoica* rust complex (i.e. *P. monoica* Arth., *P. consimilis* Ellis & Everh., *P. thlaspeos* C. Schub., and *P. holboellii* Hornem.) and indigenous to the Intermountain West (Kropp et al., 1997; Roy et al., 1998). While the true identification of the rust fungus currently remains unknown (Kropp et al., 1997), it has been labeled as *P. thlaspeos* 'woad strain' or commonly referred to as 'dyer's woad rust' (Environmental Protection Agency, 2002; Mandelbaum and Kough, 2002).

P. thlaspeos 'woad strain' was assessed as a potential biological control agent and federally approved in 2002 as a mycoherbicide, named the 'Woad Warrior' (Environmental Protection Agency, 2002; Mandelbaum and Kough, 2002). Mycoherbicides are applied annually in an augmentative approach (TeBeest, 1993), which can increase the proportion of plants infected within a population, to a range of 39% to 76% in targeted populations based on the timing of inoculation (Flint and Thomson, 2000; Kropp et al., 2002). Despite its success in the field and ability for mass production, the mycoherbicide is no longer commercially produced (Kropp et al., 2002; Thomson and Kropp, 2004; Winston et al., 2014). Nevertheless, *P. thlaspeos* 'woad strain' is well established throughout the Intermountain West and remains a viable and potential biological control agent of *I. tinctoria* (Flint and Thomson, 2000).

The incidence of *P. thlaspeos* 'woad strain' was initially assessed at the center of discovery (a 0.5 ha population) over an 8- and 9-year period (Daines, 1988; Lovic et al., 1988; Lovic, 1991) and after artificial inoculation at select locations throughout northern Utah (Flint and Thomson, 2000; Kropp et al., 2002). However, the presence and annual fluctuation in incidence of *P. thlaspeos* 'woad strain' has not been assessed in natural populations or on a larger geographic scale. Additionally, the proportion of seed reduction of rust infected plants has only been evaluated qualitatively (McConnell et al., 1997). Our objective was to assess if and how *P. thlaspeos* 'woad strain' regulates natural populations of *I. tinctoria* through quantifying 1) the presence and incidence of *P. thlaspeos* 'woad strain' in natural populations

throughout southeastern Idaho and northern Utah over two years, and 2) its effect on the reproductive output of *I. tinctoria*, at the individual plant and population level, and on a larger geographical scale.

Material and Methods

The invasive plant

In the American Intermountain West, *Isatis tinctoria* is a biennial or short-lived perennial (Farah et al., 1988). Seeds can germinate in the autumn months, following reproduction, but predominately germinate the following spring (Farah et al., 1988). Root development, rosette formation, and resource acquisition occur during the first growing season (Callihan et al., 1984; Evans, 1991). Rosettes are dormant during the winter months (Farah et al., 1988) and require vernalization, i.e. temperatures below 4 °C for 23 to 47 days, to switch from vegetative meristem to reproductive meristem (Asghari, 1993; Asghari et al., 1992). Plants resume growth shortly after snowmelt and then bolt, flower, and set seed (Farah, 1987; West and Farah, 1989). The reproductive output of an individual plant is a collection of stem and seed production. *I. tinctoria* produces 300 to 600 seeds per plant on average (Farah et al., 1988), while the highest quantity recorded exceeds 10,000 seeds per plant (McConnell et al., 1999). Seeds are dispersed from the end of July through the first snowfall (Farah et al., 1988). Seed dispersal is facilitated by humans, wildlife, wind, and rain (Callihan, 1990; Callihan et al., 1984; Farah, 1987; Farah et al., 1988; Hurry, 1930; NWCB, 1999; Pokorny and Krueger-Mangold, 2007).

The plant pathogen

Puccinia thlaspeos 'woad strain' is a systemic rust assumed to be native to North America and completes its 3-stage lifecycle (microcyclic) only on *I. tinctoria* (autoecious) (Environmental Protection Agency, 2002; Kropp et al., 1997; Mandelbaum and Kough, 2002). Teliospores germinate during spring and basidiospores infect young rosettes (Flint et al., 1993). The rust is asymptomatic for the initial 3 to 9 months following infection, overwinters in the root system of *I. tinctoria*, and affects new growth during the subsequent spring (Flint et al., 1993; Kropp et al., 1996). Plants that remain in the rosette stage the following spring show symptoms of chlorosis, leaf distortion, and stunted growth, followed

by the production of spermatia and telia on the underside of leaves (Kropp et al., 1995). The symptoms that occur on reproductive plants are dependent on the plant phenostage. During bolting, most leaves are chlorotic and bear spermigonia (Flint and Thomson, 2000). Upon maturation, reproductive stems remain vegetative with malformed leaves covered in teliosori (Kropp et al., 1995). Symptomatic plants occasionally produce flowers and viable seeds in low numbers (Lovic et al., 1988; Lovic, 1991; McConnell et al., 1997); however, reproductive structures, on the ends of inflorescences, are frequently replaced by flower-like leaves (Flint and Thomson, 2000). Teliosori can develop on the reproductive structures of symptomatic plants (flowers and flower-like leaves) to further facilitate rust reproduction (Flint and Thomson, 2000). The viability of seeds bearing teliosori is not compromised; nor is the rust vertically transmitted via seed to the next generation (Kropp et al., 2002). Occasionally, symptomatic plants contain asymptomatic reproductive stems, which appear to be unaffected by the rust (Flint and Thomson, 2000; Kropp et al., 1996).

Study area

The Bear River Basin is a watershed spanning 20,000 sq. km and is home to the Bear River, the largest tributary of the Great Salt Lake. The Basin spans across southeastern Idaho, northern Utah, and southwestern Wyoming in the Intermountain western region of the United States (Fig. 1*). I. tinctoria* infestations were found based on information provided by land managers and researchers, and discovered opportunistically during ongoing field work. Study sites were selected based on accessibility, sufficient population size and density, and whether they were unmanaged. Most study sites were hill slopes or disturbed habitats (i.e. roadsides, fallow lands, or ruderal areas) and exposed to full sunlight (Table 1). The *I. tinctoria* populations assessed were estimated to range from 146 m^2 to approximately 4,200 m^2. The spatial distribution of populations varied from dense clumps with sparse individuals connecting aggregates across the landscape (CMR3, CMR5, CMR6) to populations spanning entire hill slopes (POC, BC1). Over the course of this study, 14 natural *I. tinctoria* populations were surveyed and/or sampled throughout the Bear River Basin (Table 1).

Rust presence and incidence

In 2010, several *I. tinctoria* infestations were preliminarily assessed and monitored in the Basin for the presence of *P. thlaspeos* 'woad strain' (data not shown), and four sites (MAN2, NL, NS, and POC) were *sampled* for preliminary assessment of seed production per infection type (Table 1). Eleven *I. tinctoria* populations were chosen as *study* sites in 2011 with an addition of three populations in 2012 (two *survey*, one *sample*; Fig. 1, Table 1). In both years, all *survey* sites were non-destructively assessed for the presence and incidence of *P. thlaspeos* 'woad strain', and stem production per plant for each infection type; while all *sample* sites were destructively assessed for the number of seeds produced per stem or plant for each infection type. Non-destructive and destructive assessments were kept separate on study sites that were both surveyed and sampled in the same year.

At each site surveyed we collected information using the standard impact monitoring protocol (SIMP) (Weed and Schwarzländer, 2014, see Appendix 1). Sites were surveyed along a permanent 20 m transect placed to minimize the number of empty survey quadrats. Ten 0.25 m by 0.50 m (0.125 m^2) survey quadrats were marked every 2 m along the transect using colored construction whiskers attached to a stake nail. Rust infection was identified and determined per quadrat in the field by the presence and/or absence of plant symptoms. Upon assessment, plants were divided into three infection types: asymptomatic plants, symptomatic plants, and A/S plants. Asymptomatic plants lack any visual signs of infection (e.g. chlorosis, malformed growth, spores) and appear healthy; however, the presence or absence of rust infection in asymptomatic plants can only be confirmed using the polymerase chain reaction (PCR) and taxon-selective primers (Kropp et al., 1995). Symptomatic plants show symptoms of rust infection and typically contain rust spores. A/S plants contain both symptomless (asymptomatic) and symptomatic reproductive stems (Flint and Thomson, 2000). For the purpose of rust presence and incidence, A/S plants were categorized as symptomatic plants due to the presence of rust infection. Along each transect, we counted the number of reproductive plants for each infection type, the number of reproductive stems per plant, and the number of rosettes for each quadrat at all sites surveyed.

Stem production

The mean number of stems per plant was calculated separately for each infection type (asymptomatic, symptomatic, and A/S). One site in Pocatello, ID (data not shown), was selected to assess the number of flowering stems per plant only and was not included in any further analyses.

Environmental factors

Signs of previous disturbance events, such as construction or fire, were recorded for each survey site at the time transects were established, while signs of manual, mechanical, or chemical management were recorded prior to each annual assessment. Sites with previous disturbance (OG1) were included in statistical analyses because 1) disturbances are common and 2) the disturbance occurred in previous years, which allowed the plant population time to respond to the disturbance. Sites managed for the control of *I. tinctoria* were removed from all rust presence and incidence analyses the year treatment occurred (OG3 and BC2, 2012).

For the purpose of this study, plant density is the number of reproductive plants per site (1.25 m^2, Table 2). Plant density was divided into three density classes each year: fewer than 11 [$n = 6$], 11 to 25 [$n = 6$], and more than 25 [$n = 10$] plants per site. Only sites present with *P. thlaspeos* 'woad strain' ($n = 19$) were included in the comparative analyses between rust incidence and plant density. Rosettes were not included as a component of plant density because they occupy the understory of this system and therefore, do not interfere with the wind dispersal of rust spores to directly affect rust incidence. Survey sites were categorized by three site characteristics to assess whether these abiotic and biotic factors may influence rust incidence, plant density, and/or stem production amongst sites and between years: geography, elevation, and habitat type (Table 2). Categorization by geography was correlated with the nearest weather stations since most study sites were distributed throughout the Cache Mountain Range where geographical aspects change over a short distance (Table 3). Survey sites were divided into four groups: north of Logan, UT (1) [$n = 2$], south of Logan, UT (2) [$n = 4$], around Brigham City, UT (3) [$n = 2$], and around Ogden, UT (4) [$n = 4$]. Elevation was based on meters above mean sea level (AMSL) collected during transect establishment and divided into three groups: below 1300 m [$n = 4$], 1300 m to 1700 m [$n = 5$], and above 1700

m [*n* = 3]. Four habitat types were identified: grassland [*n* = 5], wetland [*n* = 2], shrubland [*n* = 2], and ruderal [*n* = 3].

Rust severity on seed production

The effect of *P*. *thlaspeos* 'woad strain' infection on *I*. *tinctoria* seed production was preliminarily studied in 2010. Reproductive stems were counted and collected from a mixture of 5 to 20 asymptomatic, symptomatic, and A/S plants, at each study site (MAN2, NL, NS, and POC). The number of seed pods (silicles) was counted from one reproductive stem per plant to avoid pseudo-replication. Since *I*. *tinctoria* produces one seed per pod (Al-Shehbaz, 2012), we used the number of seed pods counted as an equivalent for seed production per stem or plant. Visibly empty seed pods were removed from counts.

In 2011, reproductive stems were counted and collected from 30 asymptomatic, 30 symptomatic, and 15 A/S plants at study site OG1 (Table 1). The number of seed pods was counted from one reproductive stem per plant to avoid pseudo-replication, with the exception of A/S plants where one reproductive stem was counted per stem type. In 2012, the sampling regime was slightly modified to assess seed production per plant rather than that of individual reproductive stems. A minimum of 15 asymptomatic and 15 symptomatic plants were collected at each study site (POC, NS, NL, LOG3, BC1, and OG4; Table 1). We counted the number of reproductive stems per plant and the number of seed pods produced per reproductive stem per plant. Due to the scarcity of A/S plants in the field, A/S plants were not sought out in 2012 but were included in our analyses if they were encountered.

P. *thlaspeos* 'woad strain' infection often results in plant sterilization, i.e. the prevention of floral and seed production (Daines, 1988). To understand the frequency of plant sterilization and the severity of *P*. *thlaspeos* 'woad strain' on *I*. *tinctoria* seed production, the effect was divided into three categories: plant sterilization, seed reduction, and the overall effect (Table 4, Table 5). Seed reduction is the decreased percentage of seeds produced compared to that of an asymptomatic plant. The overall effect is the cumulative impact of plant sterilization and seed reduction on seed production of symptomatic plants. A/S plants were excluded from these analyses, which removed three of the four sites sampled in 2010 (NL, NS, and POC).

Analyses on seed production utilized asymptomatic and symptomatic plants from the six study sites sampled in 2012 only (Table 1). In 2012, we had consistently high sample sizes across sites for both infection types (i.e. a minimum of 15 plants) and seed production which was assessed by plant. Seed production of A/S plants was assessed separately and by the infection type of each stem per plant before compared to asymptomatic and symptomatic plant samples by stem. Only four sites (OG1 in 2011 and BC1, NL, and NS in 2012) were included in these comparative analyses due to the insufficient number of A/S plants encountered in 2012. To estimate the total reduction in *I. tinctoria* seed production in 2012, we combined the average number of seeds produced per infection type with the rust presence and incidence per infection type at each site surveyed. The reduction in seed production could only be estimated for 2012 due to insufficient replication of sites sampled in 2011 and because seed production was assessed by stem as opposed to by plant.

Statistical analyses

We calculated the aggregate proportion of rust incidence per survey site, i.e. the number of symptomatic plants divided by the total number of plant per site, due to the high variability of plants and rust infection per quadrat, assuming an approximate standard error of: $\sqrt{\frac{Pq}{n}}$, where P is the rust incidence per site, q is 1-P, and n is the number of plants per site (Fig. 2). All responses (i.e. rust incidence, plant density, and average stem and seed production) were analyzed using a generalized linear mixed model (GLMM) to assess the effects 1) amongst sites, 2) between years, 3) by infection type, and 4) across all interactions (Stroup, 2012; Stroup, 2015). In each assessment, an appropriate distribution and link function was selected for the response. Rust incidence was assumed to be binomially distributed with a logit function. Plant density, and stem and seed production were assumed to follow a Poisson distribution with a log function, unless greater variability in the data (overdispersion) was detected, in which case a negative binomial distribution was used with a log function (i.e. plant density and seed production). To assess the relationship between rust incidence and total plant density we used a Pearson correlation coefficient (PCC, Fig. 3). Analyses of plant density and stem production were based on the initial 11 sites surveyed for

comparison between years, which included sites managed for the control of *I. tinctoria* (site OG3, 2012).

Analyses of *P. thlaspeos* 'woad strain' infection on *I. tinctoria* seed production utilized all sites in all years (Fig. 4). Samples from A/S plants were combined with their respective infection type for each year. All sites sampled in 2010 and 2011 were assessed and analyzed by stem, while sites sampled in 2012 were by plant (Fig. 4). To assess the frequency of plant sterilization between infection types we used a χ-squared test of homogeneity. The percentage of seed reduction and the overall effect were calculated: $(1 - (\textit{mean seed production of symptomatic} / \textit{mean seed production of asymptomatic})) \times 100$. All zeroes within the data were replaced with one-one hundredth for statistical purposes. All statistical analyses were conducted using SAS® software, Version 9.4 (SAS, 2016).

Results

Rust presence and incidence

Rust presence at survey sites was 100% (n = 11) in 2011 and 77% (n = 13) in 2012 (Fig. 2). Although our survey methods did not detect *P. thlaspeos* 'woad strain' at three sites (CMR5, BC1, and OG2) in 2012, it was observed to be present elsewhere at these sites. Rust incidence, i.e. the proportion of plants infected with *P. thlaspeos* 'woad strain', differed amongst sites and between years (site: F (11, 9) = 5.68, P = 0.0073; year: F (1, 9) = 9.36, P = 0.0136, Fig. 2). The mean rust incidence amongst survey sites increased from 16.8% (± 4.4 SE, range: 2.0% to 47.8%) in 2011 to 29.0% (± 8.2 SE, 0% to 66.7%) in 2012. Across both years, survey sites with fewer than 11 plants per site (1.25 m^2) showed some of the highest levels of rust incidence and variance (mean: 40.8% ± 11.0 SE); while the higher plant density classes showed lower levels of rust incidence and variance (11 to 25 mean: 35.5% ± 9.7 SE; more than 25 mean: 16.3% ± 5.7 SE), and a negative association to rust incidence (plant density classes: F (2, 13) = 6.43, P = 0.0115; year: F (1, 13) = 5.90, P = 0.0304). When data were pooled for 2011 and 2012, there was a negative correlation between rust incidence and plant density (r = -0.5852, P = 0.0085, Fig.3). The negative relationship was not significant for the 2011 or 2012 survey site data alone (r = -0.3749, P = 0.2560; r = -0.4943, P = 0.2130, respectively). Plant density of *I. tinctoria* populations differed across sites (F (10, 10) = 3.89, P = 0.0215) and decreased between 2011 and 2012 by 67.0%, from 32.3 (± 4.3 SE) plants per

site to 10.6 (± 3.1 SE) plants per site (F (1, 10) = 44.48, $P < 0.0001$; Table 2, Fig. 2). The number of asymptomatic plants differed across sites (F (10, 10) = 4.61, $P = 0.0120$) and decreased from 27.5 (± 4.4 SE, range: 7 to 50) plants per site in 2011 to 7.3 (± 2.6 SE, range: 1 to 27) plants per site in 2012 (F (1, 10) = 52.51, $P < 0.0001$). The number of symptomatic plants varied across sites (F (10, 10) = 4.86, $P = 0.0099$) but was similar across years, 4.7 (± 1.8 SE, range: 1 to 5) plants per site in 2011, with the exception of 22 plants at survey site OG1, and 3.4 (± 1.4 SE, range: 0 to 14) in 2012 (F (1, 10) = 1.87, $P = 0.2014$, Table 2).

Stem production

The number of reproductive stems per *I. tinctoria* plant differed amongst sites and by infection type (site: F (10, 454) = 1.90, $P = 0.0432$; infection type: F (1, 454) = 12.84, $P = 0.0004$). There was a negative relationship between the number of reproductive stems per plant and plant density per site (F (2, 481) = 11.03, $p < 0.0001$), with the exception of the lowest plant density class (less than 11 plants). Asymptomatic plants had fewer reproductive stems per plant (mean 1.4 ± 0.1 SE, range: 1 to 8) than symptomatic plants (mean 2.1 ± 0.2 SE, range: 1 to 12). This pattern was significant for the 2011 survey site data alone (F (1, 340) = 16.66, $p < 0.0001$), but not the 2012 data alone (F (1, 103) = 0.15, $p = 0.6980$). One site in Pocatello, ID represented an exception to the pattern observed. In 2012, asymptomatic plants had a mean of 7.4 (± 0.8 SE) reproductive stems per plant while the highest number of stems surveyed was 25. In the absence of *P. thlaspeos* 'woad strain', no symptomatic or A/S plants were surveyed at this site. The proportion of plants that contained both asymptomatic and symptomatic reproductive stems (A/S plants) was less than 1.8% ($n = 5$) of the total number of plants surveyed throughout the study and less than 6.0% of the plants infected with *P. thlaspeos* 'woad strain', i.e. 5.8% ($n = 3$) in 2011 and 5.4% ($n = 5$) in 2012. A/S plants contained similar numbers of each stem type (F (1, 8) = 1.07, $p = 0.3313$), but had more reproductive stems per plant (mean 4.6 ± 1.2) than their respective infection classes (asymptomatic: F (1, 386) = 30.85, $p < 0.0001$; symptomatic F (1, 87) = 12.58, $p = 0.0006$).

Environmental factors

Rust incidence and plant density were categorized by three site characteristics across sites and between years: geography, elevation, and habitat type. For geography, rust

incidence differed amongst sites (F (3, 14) = 4.30, P = 0.0239). Geography 3 had the lowest mean rust incidence (4.7% ± 3.0 SE) and geography 2 had the highest (28.6 ± 7.5 SE). Both geography categories had the least amount of total precipitation across both 2011 and 2012 (Table 3, supplementary data). Rust incidence by elevation differed amongst sites and between years (sites: F (2, 16) = 7.98, P = 0.0039, years: F (1, 16) = 8.93, P = 0.0087). Survey sites between 1300 m and 1700 m had the lowest proportion of rust infected plants (16.1 ± 7.1 SE), while sites more than 1700 m in elevation had the highest (30.3 ± 6.7 SE). Rust incidence across all elevation categories increased from 2011 to 2012. For habitat type, rust incidence was similar amongst all categories. The proportion of rust infected plants was the highest at ruderal sites (30.0 ± 11.1 SE) followed by wetlands (24.6 ± 8.8 SE), grasslands (21.6 ± 8.1 SE), and shrublands (10.2 ± 5.2 SE), respectively. Rust incidence across all habitat types increased from 2011 to 2012. Comparatively, at survey sites where *P. thlaspeos* 'woad strain' was present, plant density categorized by geography differed amongst sites and between years (site: F (3, 11) = 7.61, P = 0.0050, year: F (1, 11) = 19.14, P = 0.0011). Geography 2 had the lowest mean plant density (14.6 ± 3.8 SE) and geography 3 had the highest (36.3 ± 7.4 SE). For elevation, plant density differed between years (F (1, 13) = 9.84, P = 0.0079). Plant density across different habitat types differed amongst sites and between years (site: F (3, 11) = 7.90, P = 0.0044, year: F (1, 11) = 19.00, P = 0.0011). The number of plants per site (1.25 m^2) was the highest at ruderal sites (33.3 ± 5.5 SE) followed by shrublands (29.5 ± 0.5 SE), grasslands (23.9 ± 6.9 SE), and wetlands (12.8 ± 5.2 SE). Plant density across all site characteristic subcategories decreased from 2011 to 2012. None of the interactions between survey sites and years were statistically significant for rust incidence and plant density amongst the different site characteristics ($P > 0.05$).

Categorized by geography, stem production differed amongst sites, by infection type, and the interaction amongst sites and between years (site: F (3, 477) = 3.25, P = 0.0217; infection type: F (1, 477) = 4.16, P = 0.0419; site*year: F (3, 477) = 6.72, P = 0.0002). Geography 1 had the lowest number of reproductive stems per plant (1.2 ± 0.1 SE) and geography 4 had the highest (1.7 ± 0.3 SE). Stem production by elevation differed amongst sites, by infection type, and the interaction amongst sites and between years (site: F (2, 481) = 4.81, P = 0.0086; infection type: F (1, 481) = 10.35, P = 0.0014; site*year: F (2, 481) = 12.54, $P < 0.0001$). Survey sites between 1300 m and 1700 m had the lowest stem production (1.2 ±

0.1 SE), while survey sites less than 1300 m in elevation had exceptionally high stem production (1.7 ± 0.3 SE). For habitat type, the number of reproductive stems per plant differed by infection type (F (1, 477) = 5.20, P = 0.0230). Stem production was the highest at wetland sites (1.7 ± 0.3 SE), followed by ruderal sites (1.6 ± 0.3 SE), grasslands (1.4 ± 0.1 SE), and shurblands (1.3 ± 0.2 SE). The number of reproductive stems per symptomatic plant exceeded asymptomatic plants overall and across all site characteristic subcategories. Stem production decreased across all site characteristic subcategories with the exception of geography 4 and and less than 1300 m in elevation subcategories (survey sites OG1, OG2, OG3, and OG4). Overall, stem production of asymptomatic plants was similar between years (mean: 1.4 ± 0.1 SE in 2011 and mean: 1.5 ± 0.1 SE in 2012; F (1, 381) = 0.20, P = 0.6564), while stem production of symptomatic plants decreased (mean: 2.3 ± 0.3 SE in 2011 and mean: 1.8 ± 0.3 in 2012; F (1, 82) = 2.98, P = 0.0879). None of the interactions between sites and infection type, years and infection type, and sites, years, and infection type were statistically significant for stem production amongst the different site characteristics (P > 0.05).

Rust severity on seed production

Seed production of *I. tinctoria* plants differed amongst sites, by infection type, and by the interaction between infection type within a site-year (site: F (5, 206) = 7.71, P < 0.0001; infection type: F (1, 206) = 156.69, P < 0.0001; site-year*infection type: F (5, 206) = 6.99, P < 0.0001, Fig. 4). Overall, asymptomatic stems and plants produced more seeds than symptomatic stems and plants, respectively (stem: F (1, 369) = 377.50, P < 0.0001; plant: F (1, 206) = 156.69, P < 0.0001). Although seed production was greater per asymptomatic plant than per stem (F (1, 226) = 4.04, P = 0.0455), the effect of *P. thlaspeos* 'woad strain' on *I. tinctoria* seed production was detrimental, independent of plant or stem assessment (F (1, 349) = 0.25, P = 0.6172). Asymptomatic plants produced a mean of 1,228 (± 126.3 SE, range: 28 to 6,539) seed per plant while the highest number of seeds sampled was 11,333 on one stem. In contrast, symptomatic plants produced a mean of 9 (± 5.6 SE, range: 0 to 143) seeds per plant, with an exception of 617 seeds. Seed production different by infection type amongst sites, from 600 (± 83.7 SE) to 2,069 (± 464.9 SE) seeds per asymptomatic plant (F (5, 98) = 4.46, P = 0.0011) and 0 to 43 (± 34.6 SE) seeds per symptomatic plant (F (5, 108) =

4.55, $P = 0.0008$). Seed production of A/S plants differed amongst sites, by infection type, and by the interaction between infection type within a site (infection type: F (1, 57) = 21.74, $P < 0.0001$; site: F (3, 57) = 4.93, $P = 0.0041$; infection type*site: F (3, 57) = 3.93, $P = 0.0128$). Overall, seed production of asymptomatic stems of A/S plants was similar to the stems of asymptomatic plants (F (1, 120) = 1.42, $P = 0.2357$), while the seed production of symptomatic stems of A/S plants was higher than the stems of symptomatic plants (F (1, 189) = 18.94, $P < 0.0001$).

Seed production of *I. tinctoria* infected with *P. thlaspeos* 'woad strain' was greatly diminished, if not eliminated (Table 4). *P. thlaspeos* 'woad strain' sterilized a mean of 89.5% (range: 72.2% to 100%) of plants per site (X^2 (1, $N = 218$) = 174.87, $P < 0.0001$) and reduced the 10.5% of fertile symptomatic plants by 93.6% (mean 83 ± 50.5 SE seeds produced per plant, F (1, 73) = 89.26, $P < 0.0001$). Seed reduction was similar amongst sites (F (3, 8) = 3.36, $P = 0.0758$). The combined effect of plant sterilization and seed reduction by *P. thlaspeos* 'woad strain' resulted in an overall 99.2% decrease in seed production per symptomatic plant (Table 4). *P. thlaspeos* 'woad strain' sterilized 79.5% of symptomatic stems of A/S plants overall (X^2 (1, $N = 65$) = 39.51, $P < 0.0001$), compared to 93.4% of the stems of symptomatic plants (X^2 (1, $N = 191$) = 7.06, $P = 0.0079$) and reduced the 20.5% of fertile symptomatic stems of A/S plants by 80.5% (mean 390 ± 195.3 SE seeds produced per stem, F (1, 24) = 4.48, $P = 0.0449$). Seed reduction was similar amongst sites (F (1, 6) = 0.01, $P = 0.9348$) and comparatively less than seed reduction of the stems of symptomatic plants (F (1, 25) = 6.73, $P = 0.0156$). The combined effect of plant sterilization and seed reduction resulted in a 96.1% decrease in seed production per symptomatic stem of A/S plants compared to their asymptomatic counterpart.

In the absence of *P. thlaspeos* 'woad strain', seed production of *I. tinctoria* across the 13 sites surveyed throughout the Bear River Basin in 2012 was estimated at 195,236 seeds. If plant density was similar between 2011 and 2012, we estimated seed production would have been 487,476 seeds across all sites. When the proportion of rust infected plants per site was incorporated, seed production amongst all sites was reduced by 28.9% (mean 31.7% ± 9.0 SE per site). However, when only the sites present with *P. thlaspeos* 'woad strain' were included ($n = 10$), seed production was estimated at 169,450 seeds in the absence of the rust and was reduced by 33.3% (mean 41.2 ± 9.9 SE per site).

Discussion

Rust presence and incidence

In our study, *P. thlaspeos* 'woad strain' was found in over 70% of the populations surveyed in 2011 and 2012. Rust presence varied greatly at survey sites with a density of 10 plants or less per site (1.25 m^2), which suggests there may be a minimal rust density and/or plant density threshold required to maintain the presence of *P. thlaspeos* 'woad strain' within a population. Although the rust fungus was not detected by our sampling methods at three survey sites in 2012, it was observed to be present at all survey sites in both years.

Rust incidence throughout the Bear River Basin was highly variable within individual sites, amongst sites, and between years. Rust incidence within a site is determined by whether the rust fungus has been present before, the current proportion of plants infected, rust dispersal from adjacent populations, the timing of infection, and environmental conditions (Flint and Thomson, 2000; Kropp et al., 2002). Fluctuations in rust incidence occur year to year with seasonal and annual variations in environmental conditions suitable for rust reproduction and dispersal, while variation in physical conditions can induce extreme fluctuations in rust incidence (Burdon, 1993). In both years, the mean and range in *P. thaspeos* 'woad strain' incidence amongst survey sites overlapped well with the wide variation recorded in populations during previous small scale assessments. In 1993, rust incidence was less than 1% at field sites in Preston Valley, UT and Chockcherry, UT, in 1997 it averaged 26.4% (range: 19.5% to 32.2%) across 5 sites, and in 1999 rust incidence was 0% at field sites in Park City, UT (Flint and Thomson, 2000; Kropp et al., 2002). The highest incidence of infection recorded was 75% at a field site near Trenton, UT (Kropp et al., 2002). In our study, the highest levels of rust incidence were found at study sites disturbed (i.e. construction) prior to assessment (47.8% at site OG3, 2011) and mechanically and/or chemically managed during our study (100% and 69.2% at sites OG3 and BC2, 2012, respectively).

Stem production

On average, symptomatic plants produced more stems per plant than asymptomatic plants. *P. thlaspeos* 'woad strain' infection alters morphological characteristics of *I. tinctoria*, recognized as symptoms, which facilitates rust reproduction (Flint and Thomson, 2000; Kropp

et al., 1995). Symptoms include chlorotic leaves, altered vegetative state of stems, and abnormal floral structures, which correspond with suboptimal concentrations of auxin- and cytokinin-like compounds in leaf tissues (Stirk et al., 2006). The mechanism by which hormone levels are suppressed is unknown (Stirk et al., 2006).

Environmental factors

Rust presence and incidence are a function of natural constraints driven by different aspects of the rust fungus (e.g. rust development, density and dispersal) and the host plant (e.g. host density, spatial distribution, and population size) (Burdon, 1993; Burdon and Chilvers, 1982; TeBeest, 1993). Fluctuations in rust development, spore density, and dispersal were not assessed in our study but the success of rust infection is influenced by the characteristics of host plant and rust fungus life histories, host plant genetic variation, and environmental conditions (Thrall and Burdon, 2004). *P. thlaspeos* is well synchronized to the predominantly biennial or short-lived perennial life cycle of *I. tinctoria* in the Bear River Basin (Flint and Thomson, 2000; Flint et al., 1993), but may be incapable of completing its lifecycle in other areas where *I. tinctoria* acts as a winter annual. The environmental conditions suitable for rust development, dispersal, and infection of *I. tinctoria* are conducive to spring months when rainfall is more frequent (but not required), temperatures are around 15 ºC (range: 10 ºC to 20 ºC), and young and mature rosettes occupy the understory (Flint and Thomson, 2000; Flint et al., 1993; Kropp et al., 1996; Kropp et al., 1999). Temperature and relative humidity influence the timing and duration of rust reproduction, while wind and rain influence spore density and dispersal (Flint and Thomson, 2000; Kropp et al., 2002; Kropp et al., 1999). Rust fungi may have more narrow host ranges than their host plants due to the interactions in environmental conditions (Fisher et al., 2006; Fisher et al., 2011; Fisher et al., 2008). The establishment of the *P. thlaspeos* 'woad strain' outside the Bear River Basin has not been assessed or confirmed (M. Schwarzländer, pers. comm.).

The reasons for the reductions in plant densities in 82% of survey sites in 2012 are unclear. From 2011 to 2012, the reduction in rainfall (42.3%), snowfall (48.4%), and total precipitation (46.9%) was uniform across all survey sites. Although overall plant performance and plant biomass increase with higher quantities of precipitation (Monaco et al., 2005), all survey sites received the suggested annual precipitation requirements for *I. tinctoria*

(Parker, 1975). Changes in the form or timing of precipitation may be partially responsible for this and the decrease in stem production in 2012. Nevertheless, it is difficult to identify the set of environmental factors influenced that led to the changes in plant densities and stem production across the years, and to pinpoint exactly how they were affected. For instance, reductions in any and all forms of precipitation may impact the composition and density of competitive plant species, along with the leaching of potential allelopathic chemicals from the soil. These changes can inhibit the germination and root elongation of competitive species in addition to the germination of *I. tinctoria* (Young and Evans, 1971).

The patchy distribution of rust fungi is primarily caused by the dynamics of rust dispersal (Burdon et al., 1989). In our study, *P. thlaspeos* 'woad strain' incidence increased by 58% from 2011 to 2012 as plant densities decreased by 67%. The high patchiness and variability in rust incidence and host density influences this negative relationship and may have considerable consequences at the population level (Burdon et al., 1989). At the population level, variability in rust presence and incidence can affect the size and structure of a host population through the efficacy of rust dispersal and the selective pressures exerted by the rust population. Similarly, variability in plant density and population size can have substantial demographic and genetic effects on the rust population (Burdon et al., 1989). For example, in a greenhouse study, low to mild levels of abiotic stresses (i.e. cold, dehydration, osmotic, and salinity stress) caused *I. tinctoria* plants to develop a cross-tolerance to *P. thlaspeos* 'woad strain' (Thomas and Kropp, 2011). The effects of multiple abiotic stresses on rust infection, plant fitness, and mortality have not been assessed.

Few studies have addressed the relationship between rust incidence and host plant density in nonagricultural systems. Of the cases studied, 73% were positive correlative relationships (Burdon and Chilvers, 1982). For *Cronartium ribicola* J. C. Fisch. (white pine blister rust), the density of white pine and its geographic association with its secondary host, *Ribes* spp., determined the fluctuation in rust incidence (Martin, 1944). Similarly, *P. monoica* (Pk.) Arth. incidence was greatest in populations of the Brassicaceae *Arabis holboellii* Hornem. populations when geographically associated with a population of its secondary host, *Koleria* spp., while *P. thlaspeos* C. Schub. incidence was greatest in high density populations of *Arabis holboellii* (Roy, 1993). Positive correlations between rust incidence and plant density are caused directly by increases in host density and indirectly by environmental

factors whereas negative correlative relationships are exclusively caused by indirect effects, such as temperature, relatively humidity, wind velocity, and insect vectors and facilitators (Burdon and Chilvers, 1982). Facilitated by wind, rust spores can infect individuals throughout a population as well as migrate through adjacent populations (Aylor, 1990; Flint and Thomson, 2000; Kropp et al., 2002). In tightly dense, newly infected populations (MAN2), we speculate host plants act as a wind barrier. We observed symptomatic plants only along the periphery, which limited rust dispersal to the rosettes in the immediate vicinity of the source plant. There are a few examples of negative correlative relationships between rust incidence and host plant density, such as *Cronartium fusiforme* Hedgc. & Hunt ex Cumm., fusiform rust, on southern pines (Mann and Scarborough, 1948). These relationships remain poorly understood.

Rust severity on seed production

The average number of seeds produced per asymptomatic plant greatly exceeded the average number of seeds commonly recorded for *I. tinctoria* in the area (Farah et al., 1988; McConnell et al., 1999). The only site of a similar reproductive output was POC, which had the lowest mean seed production of all sites sampled with an average of 600 seeds per plant. *P. thlaspeos* 'woad strain' is highly virulent (i.e. the consequences of infection on host plant fitness) with a severe impact on the reproductive output of *I. tinctoria*. Seed production varied across sites and with rust infection. The average number of seeds produced per symptomatic plant was consistently low across sites, regardless of plant size. Over the course of our study, over 80% of the symptomatic plants sampled were sterilized. Seed production of the remaining 20% was still substantially reduced. The overall impact of *P. thlaspeos* 'woad strain' on seed production exceeded 97% in both study years, regardless whether assessed by stem or by plant.

The severity of *P. thlaspeos* 'woad strain' on *I. tinctoria* seed production is similar to classical *Puccinia* rust fungi that often sterilize their host plants (Phatak et al., 1983; Thomas et al., 1994; Wennström and Ericson, 1991), yet is more severe than those that reduce seed production alone (Baudoin et al., 1993; Carsten et al., 2000; Emge et al., 1981; Hasan, 1974; Julien et al., 1979; Olivieri, 1984; Welch and Nelson, 1995). The novel rust fungus, *P. lagenophorae* Cooke, on *Senecio vulgaris* L. is less severe than *P. thlaspeos* 'woad strain' in

terms of plant sterilization (24% to 30%) and seed reduction (0% to 36%) (Paul and Ayres, 1986a; Paul and Ayres, 1986b), but approximately three times more severe than an old association rust, *Coleosporium tussilginis* (Pers.) Lév. (Inglese and Paul, 2006). *S. vulgaris* is more tolerant to infection by its old association, *C. tussilginis*, than *P. lagenophorae* (Inglese and Paul, 2006).

Rust virulence and host plant resistance (i.e. limitation of rust infection and fitness) and tolerance (i.e. reduction in host plant fitness consequences) drive the interactions between a rust fungus and its host plant, and inevitably, rust incidence (Roy and Kirchner, 2000). Host plant resistance has been observed through symptomless expression (Berner et al., 2015) and found infrequent in this novel plant-pathosystem during a laboratory study (Kropp et al., 1995). Host plant tolerance, in the context of plant invasions and new associations, has received little attention (Thomas et al., 1994). Symptomatic plants with asymptomatic stems (A/S plants) fully compensated for rust infection in stem and seed production. The higher stem production of A/S plants combined with the consistent or greater seed production of asymptomatic and symptomatic stems, respectively, indicates A/S plants are a form of host plant tolerance. In our study, A/S plants were relatively uncommon in natural populations (mean <10%) but most abundant at study sites where disturbance (e.g. n = 15 A/S plants sampled at site OG1, 2011) or management efforts (23% of total plants surveyed at site BC2, 2012) had occurred. If A/S plants have a considerable fitness advantage over asymptomatic and symptomatic plants, they may become more prevalent in *I. tinctoria* populations (Roy and Kirchner, 2000).

Implications for management of I. tinctoria

P. thlaspeos 'woad strain' is persistent in populations and has a severe impact on *I. tinctoria*'s reproductive output, but has low to moderate rust incidence levels across the area. Our results confirm *P. thlaspeos* 'woad strain' is widely distributed throughout the Bear River Basin but is not of epidemic status without assistance (Callihan et al., 1984; Kropp et al., 2002). Based on the severity of rust infection on seed production (99.2%), implementation of *P. thlaspeos* 'woad strain' as a novel association augmentative biological control agent has merit in areas where *I. tinctoria* has largely remained uncontrolled despite decade-long management with herbicides. In natural *I. tinctoria* populations, the annual fluctuation in

mean rust incidence can vary 1.3% to 23.2% (Kropp et al., 2002). Artificial inoculation increases spore density and expands the distribution of *P. thlaspeos* 'woad strain' throughout a population to maximize rust incidence (increase up to 22.6%) and future rust dispersal (Kropp et al., 2002). At sites with naturally high rust incidence levels, artificial inoculation has increased rust incidence to over 90% (Daines, 1988) and controlled *I. tinctoria* populations in three years (Kropp et al., 2002). However, artificial inoculation of *I. tinctoria* populations with *P. thlaspeos* 'waod strain' can be time consuming and requires annual monitoring (Kropp and Darrow, 2006; Kropp et al., 2002). Due to a lack of commercial backing, the rust is no longer mass produced, but it can be harvested from rust infected populations (Thomson and Kropp, 2004; Winston et al., 2014).

Alternatively, rust incidence could be indirectly increased through the integration of multiple management practices. Most manual volunteer control programs target reproductive plants because they are easy to spot and remove (Dorst et al., 1994; Pokorny and Krueger-Mangold, 2007). For future manual volunteer control programs, we encourage the removal of asymptomatic, symptomatic plants that produce seed, and A/S. By the removal of asymptomatic plants, host density and seed production would decrease while the proportion of rust infected plants would increase, through the increase in spore density and dispersal. Additionally, the 20% of symptomatic plants that mitigate the effects of rust infection through minimal seed production and A/S should be removed to minimize selection pressures towards rust tolerance or resistance (Roy and Kirchner, 2000). However, the disturbance associated with the removal of *I. tinctoria* plants may prevent restoration efforts through the reestablishment of *I. tinctoria* from the seed bank, the facilitation of other exotic species to invade the area, and/or the alteration of abiotic conditions (Hobbs et al., 2006; Hobbs et al., 2009).

The accumulation of natural enemies in the introduced range can insufficiently replace the species richness, abundance, prevalence, and impact (individually and cumulatively) of herbivores and pathogens compared to the native range (Keane and Crawley, 2002; Mitchell and Power, 2003; Torchin et al., 2003; Torchin and Mitchell, 2004; Wolfe, 2002). For example, specialist herbivores are often replaced by generalist herbivores in the introduced range (Joshi and Vrieling, 2005), as exotic plant species are infected by 77% fewer pathogens (Mitchell and Power, 2003). As such, accompanying the assessment of novel associations,

multiple species in the naturalized range have been assessed for the classical biological control of *I. tinctoria* (Cortat et al., 2008), with the current selection of three potential agents for release, *Ceutorhynchus rusticus* Gyllenhal, *C. peyerimhoffi* Hustache, and an undescribed eriophyid mite species in the genus *Metaculus*. *C. rusticus* is a root-mining weevil, which has been shown to reduce *I. tinctoria* biomass by 46% and seed production by 72%, and induce plant mortality in heavily attacked rosettes (H. Hinz and E. Gerber, unpubl. data). This agent alone should have a significant impact on *I. tinctoria* populations. When combined with *P. thlaspeos* 'woad strain', the interaction between these two natural enemies, as well as the overall effect on *I. tinctoria* populations, is unknown. The interaction and overall effect is dependent on the timing and sequence of these natural enemies, and the host plant responses (Hatcher et al., 1994). For example, the changes in auxin- and cytokinin-like compounds induced by *P. thlaspeos* 'woad strain' may deter *C. rusticus* or negatively impact weevil biomass and fecundity (Turner et al., 2010). On the contrary, *C. rusticus* may interact similar to the oligophagous shoot-base boring weevil, *Apion onopordi* Kirby on *Cirsium arvense* (L.) Scop., where *A. onopordi* facilitates rust dispersal and *P. punctiformis* (Str.) Röhl. indirectly increases weevil survival and fecundity (Bacher et al., 2002; Friedli and Bacher, 2001). Than again, the stress of *C. rusticus* on the root system may weaken *I. tinctoria* rosettes and the added stress of rust infection may cause plant mortality. Although the relationship between these natural enemies may be antagonist, they should have a positive cumulative effect on *I. tinctoria* populations.

C. peyerimhoffi has been shown to decrease seed production of *I. tinctoria* plants through adult feeding and larval mining by up to 98.5% (Hinz et al., 2016). *I. tinctoria* populations in the Intermountain West are the result of 3% of the total number of seeds produced annually (Farah et al., 1988). *C. peyerimhoffi* is not likely to have a significant impact on *I. tinctoria* populations alone unless it can maintain the densities to reduce the average seed production by 97% continuously (Crawley, 2000; Myers and Risley, 2000; Olckers, 2011; Paynter et al., 1996; van Klinken and Flack, 2008; van Klinken et al., 2004). However, *C. peyerimhoffi* should have a greater effect on *I. tinctoria* populations when combined with *P. thlaspeos* 'woad strain' through the reduction on seed production of asymptomatic plants, and hopefully on the 20% of symptomatic plants with minimal seed production, and A/S plants.

Assessments on the interactions between and the overall effects of the three biological control candidates with *P. thlaspeos* 'woad strain' prior to release of the insects are logistically difficult. However, we strongly encourage the interactions and overall effects be assessed in quarantine prior to release. The recorded impact of these natural enemies in the native range may not compare to what we may observe in the introduced range because in our study, the average seed production of *I. tinctoria* plants was 2.5–fold higher than plants in central Europe (H. Hinz, pers. comm.). Nevertheless, based merely on the impact of the natural enemies and the characteristics of the host plant, rust fungus, and insect's life histories, we speculate moderate interspecific competition between *P. thlaspeos* 'woad strain' and *C. rusticus*, minimal interspecific competition between *P. thlaspeos* 'woad strain' and *C. peyerimhoffi*, but a positive cumulative impact from each of these combinations (Denoth et al., 2002).

P. thlaspeos 'woad strain' alone is unable to halt the spread and reduce the density of *I. tinctoria* invasions. Its incidence can be increased through artificial inoculation and/or the conservation of infected plants. Should additional novel associations be discovered and/or classical biological control agents be released, we suspect these will have an overall beneficial effect when combined with the rust fungus on the control of *I. tinctoria* populations. *P. thlaspeos* 'woad strain' is a permanent component of the *I. tinctoria* ecosystem and an additional tool for current and future management practice.

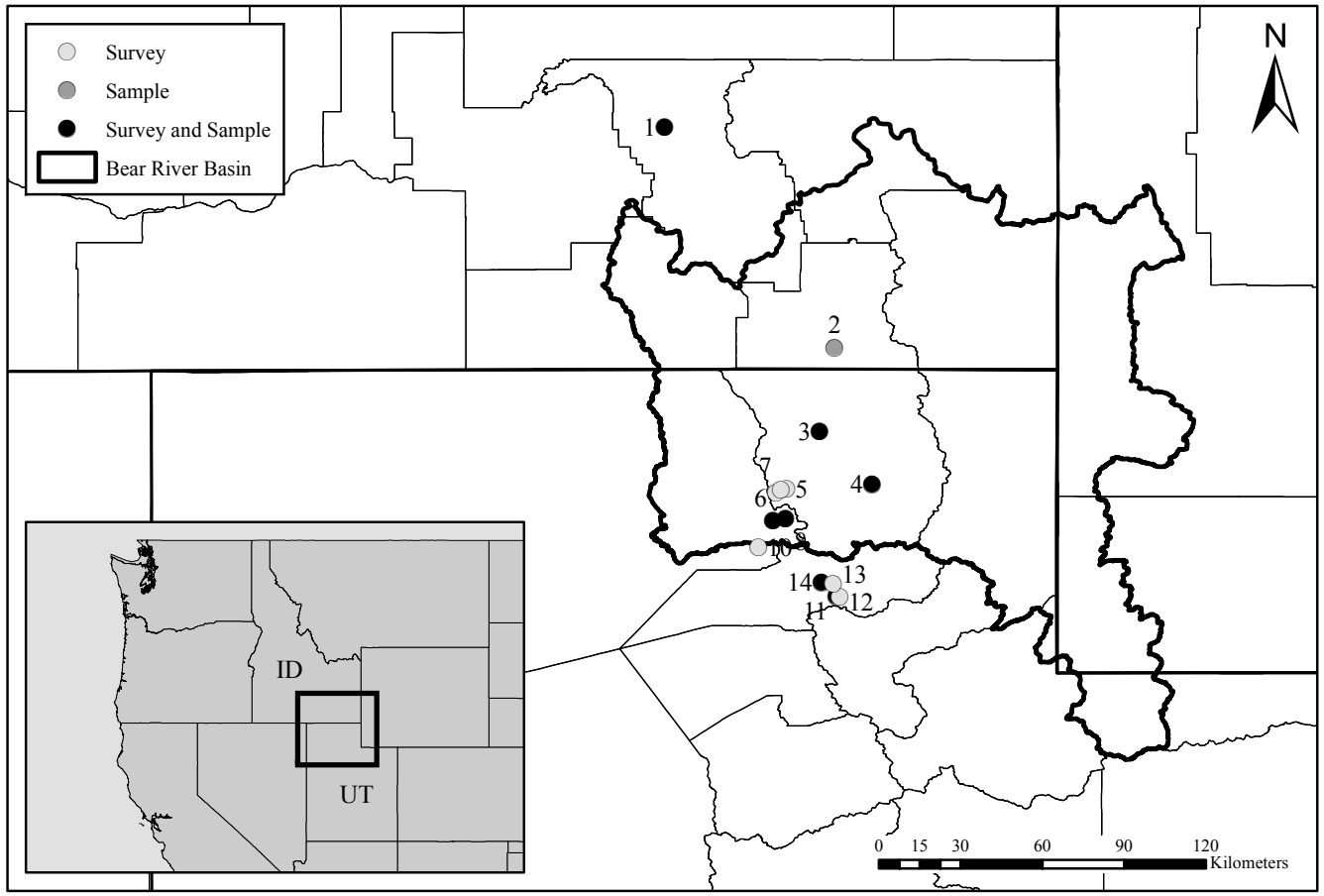

Figure 1: Locations of *Isatis tinctoria* study sites surveyed and sampled for *Puccinia thlaspeos* 'woad strain' infection in the Bear River Basin area in southeastern Idaho and northern Utah, USA. White circles, study sites strictly sampled for rust severity on seed production in 2012; gray circles, study sites strictly surveyed for rust presence and incidence and stem production in 2011 and/or 2012; black circles, study sites surveyed for rust presence and incidence and stem production, and sampled for rust severity on seed production in 2011 and/or 2012.

Table 1: *Isatis tinctoria* study sites surveyed and/or sampled for *Puccinia thlaspeos* 'woad strain' (rust) infection in the Bear River Basin area in southeastern Idaho and northern Utah, USA. Sites were non-destructively *surveyed* for the presence and incidence of rust infection and the number of stems per plant, while sites were destructively *sampled* for the number of seeds produced per stem (2010 and 2011) or per plant (2012).

Site #	Site name	City	State	Coordinates (DDM)		Survey year(s)		Sample year(s)			Patch size (ha)
1	POC	Pocatello	Idaho	N 42°47.919'	W 112°20.719'		2012	2010		2012	
2	NS	Preston	Idaho	N 42°04.285'	W 112°47.046'			2010		2012	
3	NL	Logan	Utah	N 41°47.694'	W 111°50.030'	2011	2012	2010		2012	0.092
4	LOG3	Hyrum	Utah	N 41°37.210'	W 111°39.646'	2011	2012			2012	0.022
5	CMR3	Logan	Utah	N 41°36.322'	W 111°56.590'	2011	2012				0.055
6	CMR5	Mantua	Utah	N 41°35.609'	W 111°56.663'	2011	2012				0.028
7	CMR6	Mantua	Utah	N 41°36.245'	W 111°57.873'	2011	2012				0.038
8	MAN2	Mantua	Utah	N 41°30.433'	W 111°56.749'	2011	2012	2010			0.080
9	BC1	Brigham City	Utah	N 41°30.019'	W 112°59.235'	2011	2012			2012	< 0.428
10	BC2[b]	Brigham City	Utah	N 41°14.859'	W 112°01.299'		2012				
11	OG1[a]	Ogden (Eden)	Utah	N 41°14.953'	W 111°46.677'	2011	2012		2011		0.034
12	OG2	Ogden (Eden)	Utah	N 41°14.832'	W 111°46.048'	2011	2012				0.015
13	OG3[b]	Ogden (Eden)	Utah	N 41°17.522'	W 111°47.350'	2011	2012				0.048
14	OG4	Ogden (Eden)	Utah	N 41°17.761'	W 111°49.583'	2011	2012			2012	0.076

[a] Study sites with previous disturbance.
[b] Study sites manually, mechanically, and/or chemically managed for the control of *Isatis tinctoria*.

Table 2: The percentage of *Isatis tinctoria* plants infected with *Puccinia thlaspeos* 'woad strain', site characteristics, and the number of plants per site/1.25 m^2 (plant density) at survey sites in southeastern Idaho and northern Utah in 2011 and 2012.

			Site characteristics			Plant density (1.25 m^2)		
Year	Site name	Rust incidence (%)	Geography	Elevation (m)	Habitat type	Total	Asymptomatic	Symptomatic
2011	NL	12.1	1	1701	Ruderal	33	29	4
2011	LOG3	21.7	2	1753	Wetland	23	18	5
2011	CMR3	30.0	2	1495	Grassland	10	7	3
2011	CMR5	12.5	2	1540	Grassland	32	28	4
2011	CMR6	31.3	2	1785	Grassland	16	11	5
2011	MAN2	2.0	3	1472	Ruderal	51	50	1
2011	BC1	13.3	3	1451	Shrubland	30	26	4
2011	OG1[a]	47.8	4	1281	Ruderal	46	24	22
2011	OG2	2.0	4	1281	Grassland	50	49	1
2011	OG3	2.3	4	1281	Grassland	44	43	1
2011	OG4	10.0	4	1282	Wetland	20	18	2
2012	POC	17.2	1	1415	Shrubland	29	24	5
2012	NL	50.0	1	1701	Ruderal	28	14	14
2012	LOG3	16.7	2	1753	Wetland	6	5	1
2012	CMR3	66.7	2	1495	Grassland	3	1	2
2012	CMR5	0.0	2	1540	Grassland	1	1	0
2012	CMR6	50.0	2	1785	Grassland	12	6	6
2012	MAN2	3.6	3	1472	Ruderal	28	27	1
2012	BC1	0.0	3	1451	Shrubland	2	2	0
2012	BC2[b]	69.2	3	1279	Ruderal	13	4	9
2012	OG1[a]	64.3	4	1281	Ruderal	14	5	9
2012	OG2	0.0	4	1281	Grassland	18	18	0
2012	OG3[b]	100.0	4	1281	Grassland	3	0	3
2012	OG4	50.0	4	1282	Wetland	2	1	1

[a] Study sites with previous disturbance.

[b] Study sites manually, mechanically, and/or chemically managed for the control of *Isatis tinctoria*.

Table 3: Weather data extrapolated to survey sites in southeastern Idaho and northern Utah in 2011 and 2012.

					Annual precipitation (cm)		
Year	Geography	Weather station	Coordinates (DDM)		Rainfall	Snowfall	Total
2011	1	Logan, Utah State University	N 41°44.760’	W 111°48.180’	68.6	250.2	318.8
2011	2	Logan, 5 SW Experimental Farm	N 41°39.960’	W 111°53.460’	57.4	162.3	219.7
2011	3	Brigham City, Waste Plant	N 41°31.440’	W 112°02.640’	51.6	170.2	221.8
2011	4	Ogden, Pine View Dam	N 41°15.480’	W 111°50.280’	113.7	308.6	422.3
2012	1	Pocatello, Regional Airport	N 42°55.200’	W 112°34.260’	26.1	40.1	66.2
2012	1	Logan, Utah State University	N 41°44.760’	W 111°48.180’	36.4	130.0	166.4
2012	2	Logan, 5 SW Experimental Farm	N 41°39.960’	W 111°53.460’	36.4	97.8	134.2
2012	3	Brigham City, Waste Plant	N 41°31.440’	W 112°02.640’	34.4	84.8	119.2
2012	4	Ogden, Pine View Dam	N 41°15.480’	W 111°50.280’	60.8	147.3	208.1

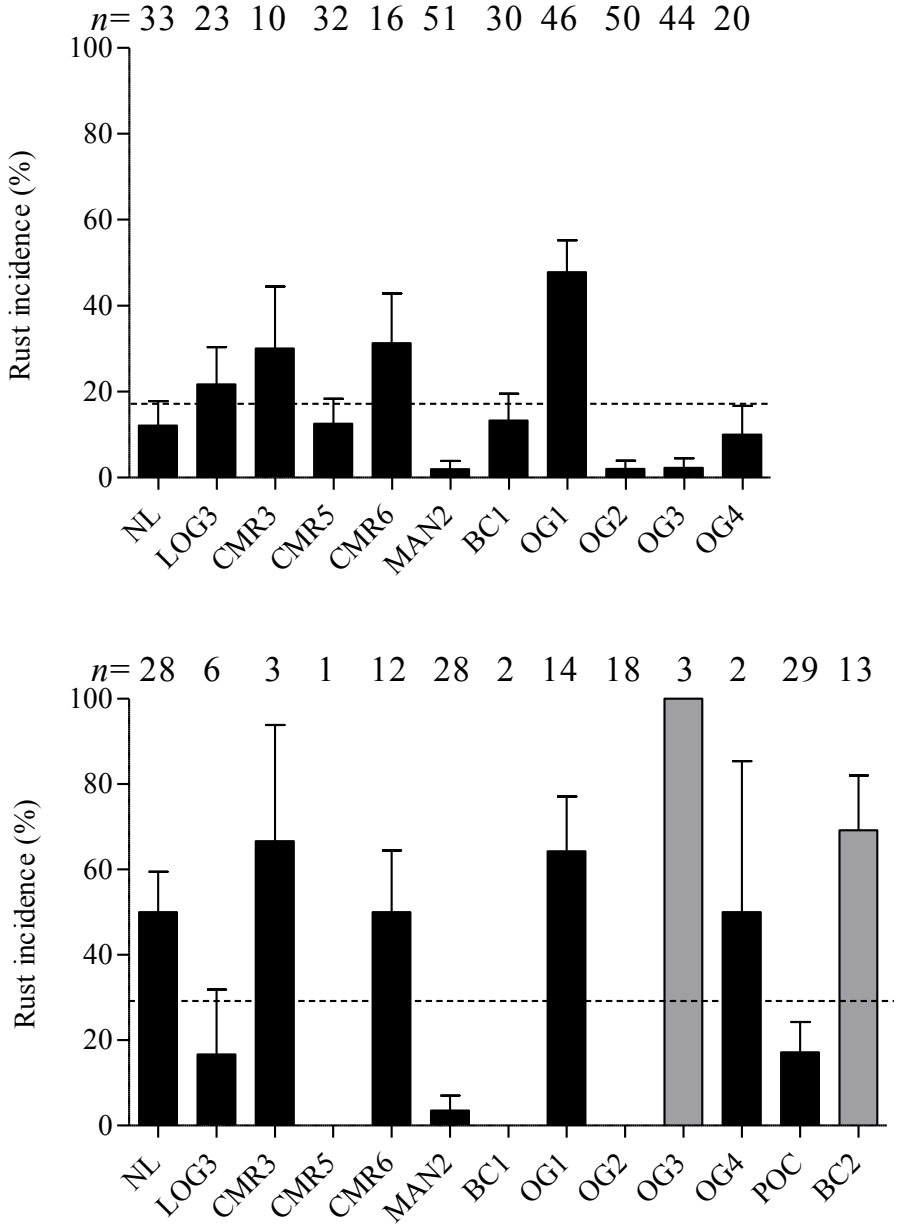

Figure 2: The presence and percentage of *Isatis tinctoria* plants infected with *Puccinia thlaspeos* 'woad strain' at survey sites in southeastern Idaho and northern Utah in 2011 (top graph) and 2012 (bottom graph). Sites with gray columns (OG3 and BC2) were managed for the control of *I. tinctoria* and therefore, excluded from analyses for rust incidence. Error bars denote the approximate standard error for binomial proportions; *n*=, number of plants per site/1.25 m^2 (plant density).

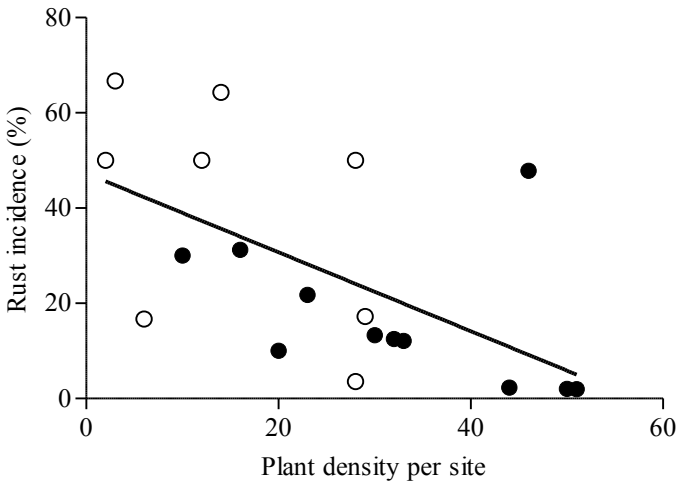

Figure 3: Relationship between the percentage of *Isatis tinctoria* plants infected with *Puccinia thlaspeos* 'woad strain' and the average number of plants per site/1.25 m^2 in southeastern Idaho and northern Utah in 2011 (black circles) and 2012 (white circles) (r = -0.5852, P = 0.0085; n = 11 in 2011 and n = 8 in 2012). Survey sites absent of rust according to our survey methods or managed for the control of *I. tinctoria* in 2012 were excluded.

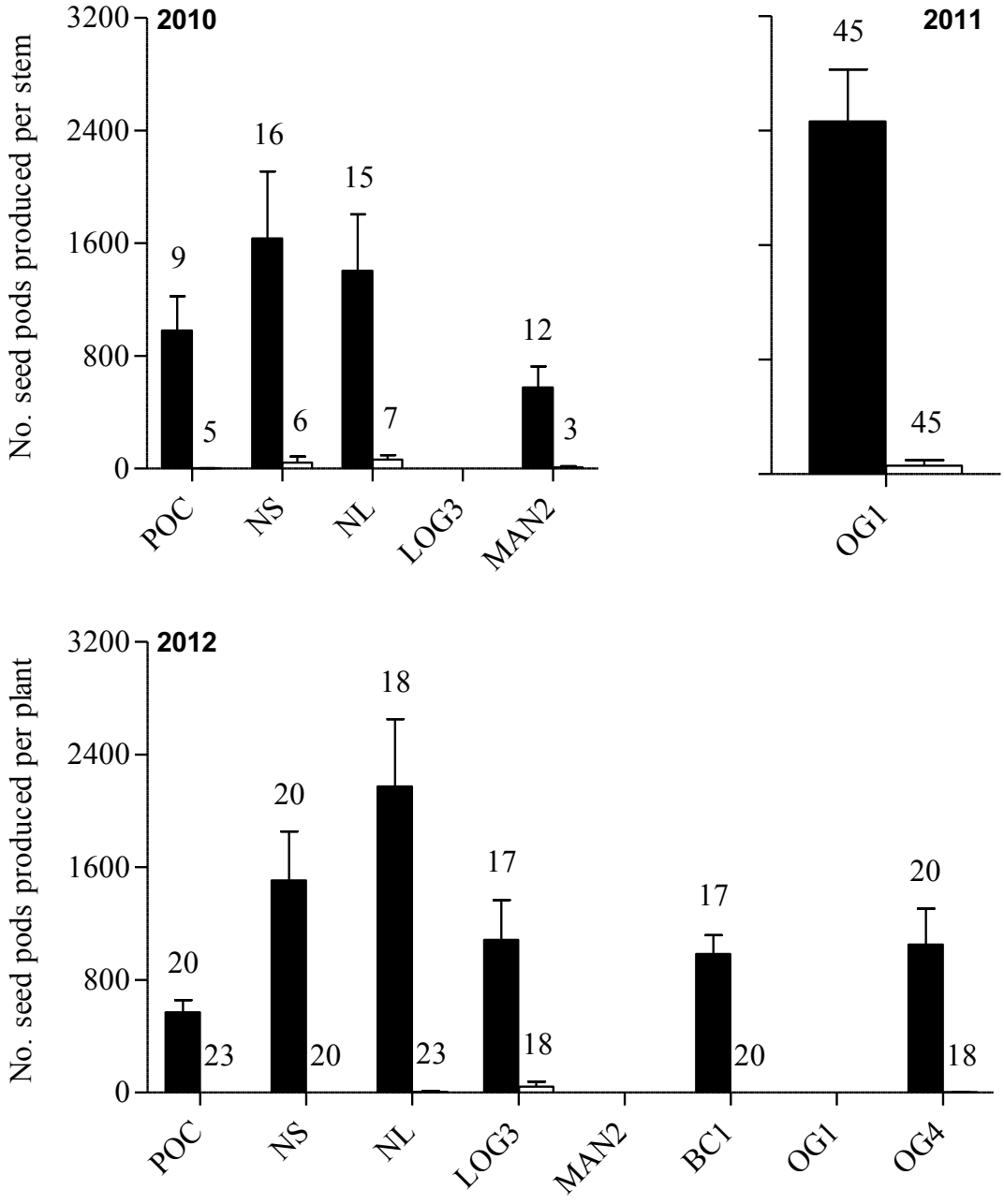

Figure 4: The mean (± SE) number of seed pods produced by asymptomatic (black bars) and symptomatic (white bars) *Isatis tinctoria* reproductive stems (top graphs) and plants (bottom graph) sampled between 2010 and 2012 (site-year: F (10, 375) = 10.74, $P < 0.0001$, infection type: F (1, 375) = 356.56, $P < 0.0001$, site-year*infection type: F (10, 375) = 6.87, $P < 0.0001$). Numbers above bars represent sample sizes.

Table 4: The effect of *Puccinia thlaspeos* 'woad strain' on *Isatis tinctoria* seed production by stem (2010 and 2011) and plant (2012).

Year	% symptomatic stems/plants sterilized[a]	% seed reduction on symptomatic stems/plants[b]	Overall effect of sterilization and % seed reduction on symptomatic stems/plants[c]
2010	66.7 ± 0.0[d]	95.5 ± 0.0[d]	98.5 ± 0.0[d]
2011	76.7 ± 0.0[d]	98.4 ± 0.0[d]	99.6 ± 0.0[d]
2012	89.1 ± 4.8	94.2 ± 3.1	99.2 ± 0.6

[a] The percentage of symptomatic stems or plants that fail to produce seeds (86.4% overall).
[b] The percentage of seed production reduced on symptomatic stems or plants compared to their respective asymptomatic stems and plants.
[c] The combined percentage of plant sterilization and seed reduction on the seed production of symptomatic stems or plants.
[d] Only one site assessed.

Table 5: The statistical significance of *Puccinia thlaspeos* 'woad strain' on *Isatis tinctoria* seed production by stem (2010 and 2011) and plant (2012).

	% seed reduction on symptomatic stems/plants			Overall effect of sterilization and % seed reduction on symptomatic stems/plants		
Source	Df	*F*	*P*	Df	*F*	*P*
Site-year	5	3.88	0.0027	7	6.32	< 0.0001
Infection type	1	127.67	< 0.0001	1	209.41	< 0.0001
Site-year*infection type	5	3.22	0.0091	7	5.09	< 0.0001

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