Effects of Brachypterolus pulicarius on growth and seed production of Dalmation toadflax
by Robert Thomas Grubb

A thesis submitted in partial fulfillment of the requirements for the degree Master of Science in Entomology
Montana State University

Abstract:
The impact of the ovary-feeding beetle Brachypterolus pulicarius (L.) (Coleoptera: Nitidulidae) on the growth and reproduction of Dalmatian toadflax, Linaria genistifolia spp. dalmatica Maire and Petitmengen, was evaluated through greenhouse and field studies. A hand pollination study was conducted to. determine the pollination technique which achieved maximum fertilization of Dalmatian toadflax flowers. This study was a prerequisite in evaluating the beetle's impact on Dalmatian toadflax reproduction. Results of analysis of variance on seed capsule production and volume suggest that hand pollinating flowers twice within the first five days of bloom will ensure the highest percent fertilization (72-100%) and largest seed capsule volume (29-46 mm3).

Paired-plant inclusion studies (with and without B. pulicarius) were conducted in the greenhouse and field on plants from three different age groups (three, six, and 12 months old). Experiments were replicated eight times and arranged in a split-plot design. Results of both split-plot analysis of variance and path coefficient analysis indicate that B. pulicarius feeding 1) reduced the height of Dalmatian toadflax plants up to 23 cm, 2) increased the number of primary and secondary branches by 77 and 95 %, respectively, 3) reduced the number of flowers produced per plant by 44 to 49%, and 4) decreased seed production by 43 to 93 %.

Isozyme analysis, using starch gel electrophoresis, was conducted on populations of B. pulicarius collected from either Dalmatian toadflax or yellow toadflax, Linaria vulgaris (L.) Mill, to investigate the possible existence of host races in B. pulicarius. Significant allelic frequency differences were found between beetle populations associated with different host plants. The genetic distance dendrogram based on Nei's genetic distances showed that the beetle populations generally grouped by host plant, with the exception of one population of B. pulicarius collected from yellow toadflax which grouped with other beetle populations collected from Dalmatian toadflax. Similar results were obtained using correspondence of analysis of the allelic frequencies and further support the possible existence of host races in B. pulicarius.

EFFECTS OF *Brachypterolus pulicarius* ON GROWTH AND SEED PRODUCTION OF DALMATIAN TOADFLAX

by

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ABSTRACT

The impact of the ovary-feeding beetle *Brachypterolus pulicarius* (L.) (Coleoptera: Nitidulidae) on the growth and reproduction of Dalmatian toadflax, *Linaria genistifolia* spp. *dalmatica* Maire and Petitmengen, was evaluated through greenhouse and field studies. A hand pollination study was conducted to determine the pollination technique which achieved maximum fertilization of Dalmatian toadflax flowers. This study was a prerequisite in evaluating the beetle's impact on Dalmatian toadflax reproduction. Results of analysis of variance on seed capsule production and volume suggest that hand pollinating flowers twice within the first five days of bloom will ensure the highest percent fertilization (72-100%) and largest seed capsule volume (29-46 mm^3).

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Isozyme analysis, using starch gel electrophoresis, was conducted on populations of *B. pulicarius* collected from either Dalmatian toadflax or yellow toadflax, *Linaria vulgaris* (L.) Mill, to investigate the possible existence of host races in *B. pulicarius*. Significant allelic frequency differences were found between beetle populations associated with different host plants. The genetic distance dendrogram based on Nei's genetic distances showed that the beetle populations generally grouped by host plant, with the exception of one population of *B. pulicarius* collected from yellow toadflax which grouped with other beetle populations collected from Dalmatian toadflax. Similar results were obtained using correspondence of analysis of the allelic frequencies and further support the possible existence of host races in *B. pulicarius*.

CHAPTER 1

LITERATURE REVIEW

Introduction

According to Ross (1985), out of the estimated 250,000 plant species in the world, three percent or 8,000 species are considered weeds. Of these, around 250 species, or 0.1% of the total taxa, are major problems in worldwide agriculture (Radosevich and Holt 1984, Ross and Lembi 1985). Plants that are recognized as weeds, or plants with weedy traits, typically possess certain characteristics that set them apart from other plants (Ross 1985). Some of the "ideal weed characteristics" discussed by Baker (1974) and other authors are presented in Table 1. Many plants with one or more of these characteristics can become invasive, vigorous, and competitive when introduced into areas where they are not native. Some weeds can significantly impact native or beneficial plant communities and alter animal habitat (Baker 1974, Tyser and Key 1988). The more aggressive of these non-native plants are commonly called noxious weeds.

The State of Montana, through legislative action, has defined a noxious weed "as any exotic plant species established or that may be introduced in the state which may render land unfit for agriculture, forestry, livestock, wildlife, or other beneficial uses or that may harm native plant communities" (MCA 7-22-2101 through 2153). Noxious weeds have the potential to 1) diminish soil and water resources (Lacey et al.

1989), 2) reduce biodiversity (Tyser and Key 1989, Randall 1996), 3) decrease wildlife habitat and livestock forage production (Spoon et al. 1989, Thompson 1996), and 4) alter the functioning of the ecosystem (Tandall 1996).

Currently, fifteen plant species in Montana are designated as state-wide noxious weeds. They cost Montana landmanagers millions of dollars annually in weed control measures and crop/forage losses (Nowierski et al. 1987, Lacey 1989). For example, French and Lacey (1983) calculated that spotted knapweed costs the Montana range livestock industry approximately $4.5 million per year in forage loss. This figure could potentially reach $155 million annually if spotted knapweed spread to infest all the susceptible land within the state (Bucher 1984). Today the direct impact of spotted knapweed infestations in Montana is estimated to be around $11 million per year (Hirsch and Leitch 1996).

Dalmatian toadflax, *Linaria genistifolia* ssp. *dalmatica* (L.) Maire and Petitmengin (Scrophulariaceae), is a category I noxious weed in the state of Montana. This plant possesses several of Baker's "ideal weed characteristics" including: discontinuous seed germination and longevity, cross-pollination by unspecialized visitors, high seed output, and the ability to reproduce vegetatively (Robocker 1970, 1974, Saner 1991). Since its introduction to North America in the late 1800's, Dalmatian toadflax has spread to infest much of the northwestern region of the United States and southwestern Canada (Robocker 1970, 1974). In Montana, results from a 1993 statewide survey of weed infestations indicated that Dalmatian toadflax infests over 150,000 acres (Cooksey 1993).

Dalmatian toadflax can be very aggressive in disturbed sites and is often difficult to control (Robocker 1974). This plant can also establish under intense competition (Saner 1991), but it usually does not compete well at these sites (Nowierski 1995). Losses in rangeland production by Dalmatian toadflax may be sustained through direct competition and/or reduced quality and palatability of the forage as the density of toadflax increases (Lange 1958, Reed and Hughes 1970, Robocker 1974). Mature Dalmatian toadflax plants compete intensively for soil moisture and displace native vegetation (Robocker 1974, Saner 1991). In a study of the competitive ability of mature Dalmatian toadflax, the dry weight of beneficial vegetation (92% perennial grasses and 8% winter annual forbs and grasses) decreased by 53% as the density of Dalmatian toadflax increased from the lowest to the highest density (Robocker 1974).

History and Distribution of Dalmatian Toadflax

Dalmatian toadflax is native to the northern Mediterranean region of Europe (Robocker 1970, 1974). Its range extends from the Dalmatian coast of Yugoslavia northeastward into Romania and southeastward into the countries surrounding the Black Sea from Turkey to Bulgaria. Dalmatian toadflax is also well-established in the northern regions of Syria, Iraq, and Iran (Alex 1962, Robocker 1974, Nowierski 1995).

Dalmatian toadflax has been cultivated as an ornamental plant in Europe since 1594. It was apparently brought to North America around 1894 for these same purposes (Alex 1962, Nowierski 1995). The earliest recorded wild specimen of

Dalmatian toadflax in the United States was collected in the San Gabriel Mountains of southern California in 1920 (Robocker 1974). Documented specimens of Dalmatian toadflax were also collected in the states of Maine and New York in 1922 and 1924, respectively (Alex 1962). In 1926, Dalmatian toadflax was located along the Little Spokane River northwest of Spokane, Washington (Lange 1958, Robocker 1974). This infestation expanded into northeastern Washington and northern Idaho (Lange 1958). By 1962, 15 states reported infestations of Dalmatian toadflax (Alex 1962). Presently Dalmatian toadflax is located in all states west of the 100th meridian with the exception of New Mexico. Dalmatian toadflax is also established throughout the northcentral and northeastern regions of the United States, as well as six Canadian provinces (Robocker 1974, Figure 1).

Dalmatian toadflax was first reported in southcentral Montana along a railroad siding in the 1940's. By 1980, twenty-four Montana counties had reported infestations of this plant. Dalmatian toadflax has continued to spread, and by 1993, forty-three out of the fifty-six Montana counties reported Dalmatian toadflax infestations (Forcella and Harvey 1981, Figure 1).

Taxonomy and Morphology of *Linaria genistifolia* ssp. *dalmatica*

Annual flower stems of Dalmatian toadflax are more or less woody below, ascending to erect, and range in diameter from 5-11 mm. Flowering stems rarely branch near the base, but frequently branch above (Alex 1962). Thirty to forty succulent non-flowering stems are produced in the fall. The non-flowering stems are

decumbent to weakly ascending, and generally die back in the spring after the flowering stems elongate (Alex 1962, Robocker 1974).

Dalmatian toadflax foliage is characterized as both glabrous and glaucous. Leaves of Dalmatian toadflax are alternate and can vary greatly in size and shape along the stem and from plant to plant. Alex (1962) describes the leaves of Dalmatian toadflax as reflexed to ascending, entire, sessile, occasionally concave, somewhat coriaceous, occasionally slightly rugose, acute to briefly acuminate at the tip, obtuse to cordate or obliquely cordate at the base, usually amplexicaul, with 3-7 longitudinal veins. The lower leaves tend to be linear to lanceolate (15-47 mm long, 2-11 mm wide), the middle leaves linear-lanceolate to broad-ovate (20-72 mm long, 8-42 mm wide), and the upper leaves lanceolate to very broad-ovate (30-60 mm long, 12-34 mm wide) (Alex 1962).

The inflorescence of Dalmatian toadflax comprise one or more simple racemes. Inflorescences tend to be loosely arranged, erect or nodding. Length of the inflorescence along the main stem ranges from 15-55 cm and from 12-40 cm along the upper branches (Alex 1962). Pedicels are green to red with lower pedicels 5-14 mm long and upper pedicels 3-8 mm long (Alex 1962). The calyx is made of five distinct lanceolate to ovate-lanceolate sepals 6-11 mm long and 2-4 mm wide. The bright yellow, snapdragon-like flowers are 33-55 mm long, bearded, and have a dark orange palate (Alex 1962). Flowers of Dalmatian toadflax have a tapered spur 12-18 mm long, a bilobate upper lip, and a trilobate lower lip (Alex 1962). Flowers are self-

incompatible and rely mainly on bees for cross pollination (Robocker 1974, Saner 1991).

Seed production is possible within three and one-half months after seedling emergence (Saner 1991). Seeds are produced in two-chambered ovoid capsules 4-10 mm long and 4-8 mm in diameter (Alex 1962). Each capsule can contain up to 300 slightly winged seeds 0.7-2 mm long and 0.5-1.0 mm wide (Alex 1962, Robocker 1970). A flowering stem can contain up to 30 seed pods (Robocker 1970).

Dalmatian toadflax develops an extensive root system. The vertical taproots of Dalmatian toadflax are enlarged near the root crown, woody, and contorted. Taproots can grow to a depth of 260 cm (Alex 1962, Robocker 1974). In addition to the primary vertical root, Dalmatian toadflax can produce secondary lateral roots 8.5-20 cm under the soil surface. Lateral roots can grow up to 365 cm (12 ft) from the original plant with new buds arising from 7-90 cm along the root system (Lange 1958). New shoots can be produced from root buds on both the primary and secondary root systems (Saner 1991).

Life History of Dalmatian Toadflax

Dalmatian toadflax is a short-lived perennial forb belonging to the Scrophulariaceae family (Alex 1962, Saner 1991, Nowierski, 1995). The weed is adapted to cool-semiarid climates and coarse-textured soils. Toadflax grows well in soils which vary in texture from sand to gravelly loams and range in soil pH from 6.5 to 8.5 (Alex 1962, Robocker 1974, Saner 1991).

Germination success of Dalmatian toadflax is generally low and is best in a variable environment (Alex 1962, Saner 1991). Germination occurs in the field from a depth of 2-2.5 cm below the surface (Robocker 1970). Seeds can germinate in autumn, but most seeds germinate in spring (Robocker 1970, 1974). Dalmatian toadflax seeds can germinate over a wide range of temperatures, and remain viable in the soil for over 10 years (Alex 1962, Robocker 1970, 1974, Saner 1991).

Dalmatian toadflax seedlings are not effective competitors for soil moisture with established perennials or quickly maturing winter annuals. Seedling establishment most often occurs in disturbed soils and depleted rangelands (Robocker 1974). Once established, seedlings of Dalmatian toadflax show diverse growth responses to differing environmental conditions (Robocker 1974). After seed germination, Dalmatian toadflax generally develops one primary upright flowering stem and up to three secondary flowering stems (Robocker 1974, Saner 1991). Flowering stems seldom exceed 40 cm in height and develop numerous flower buds within the first growing season (Robocker 1974). During the late spring and summer months, adventitious root buds develop along both the vertical and lateral roots. In autumn, when growth of the floral stems and flower production ceases, the root buds elongate and emerge to form a fall rosette comprising of several non-flowering stems (Alex 1962, Robocker 1974). Fall rosettes seldom emerge before September, tolerate freezing temperatures, and remain functional for varying periods of time in the spring (Robocker 1970, 1974). Fall rosettes generate carbohydrate reserves for the roots which may provide Dalmatian toadflax a competitive advantage in early spring (Robocker 1970, 1974). A second

year plant can produce up to 25 flowering stems which can range in height from 40-205 cm (Alex 1962, Robocker 1974, Saner, 1991).

Dalmatian toadflax flowers throughout the summer and into the fall (Robocker 1970). Depending on soil and environmental conditions, a large plant can produce 500,000 seeds per year (Robocker 1970, 1974). Most of the seeds (97%) are produced over a five week period during the months of June and July. Dalmatian toadflax has an upright calyx and seed capsule which prevents seeds from being released easily. Often seeds remain inside the fruits until spring. In many cases, exposure to strong winds are needed to release all of the seeds within the fruit (Saner 1991). Dalmatian toadflax seeds are easily spread by wind, water, livestock, and wildlife (Saner 1991).

A Dalmatian toadflax plant has an average life span of three to five years (Robocker 1974). The life of a Dalmatian toadflax stand varies and depends heavily upon plant competition and interaction with environmental factors (Robocker 1974, Nowierski 1995). A stand may disappear in three years under severe competition, but development of floral stems from secondary crown points along lateral roots, and the survival of new seedlings, can extend the life of the stand or allow it to expand in size and increase in density in areas of lower competition (Lange 1958, Robocker 1974).

Dalmatian Toadflax Control Measures

Chemical Control

Herbicides have been the main tool used to control Dalmatian toadflax. Picloram (4-amino-3,5,6-trichloropicolinic acid) applied to Dalmatian toadflax in

southcentral Montana provided 98% control three years after application (Sebastian and Beck 1990). Dicamba (3,6-dichloro-o-anisic acid) and tank mixes of picloram plus 2,4-D (2,4-dichlorophenoxyacetic acid) applied pre-bloom or in the fall provided between 90 to 100% control one year after application (Sebastian and Beck 1990). Both reinvasion and dormant seed germination of Dalmatian toadflax usually follow three to four years after herbicide application, making it necessary to retreat an infestation periodically for up to twelve years to achieve control (Sebastian and Beck 1990).

Cultural Control

Dalmatian toadflax does not establish well in cultivated systems, and repeated cultivation over time can effectively control this weed (Robocker 1974). Cultivation should start in early June, and continue every seven to ten days throughout the first growing season. Control of a stand requires at least two years, with four to five cultivations in the second year (Lajeunesse et al. 1993).

Burning is not considered to be an effective tool for managing Dalmatian toadflax. The soil temperatures reached by burning are usually not sufficient to kill the root buds or buried seeds (Lajeunesse et al. 1993).

Maintaining a stand of beneficial plant species can reduce Dalmatian toadflax seedling establishment. Dalmatian toadflax seedlings are poor competitors for soil moisture, and do not establish well in closed systems (Robocker 1974). Proper grazing practices may increase the health of the beneficial plant community and help reduce the

ability of Dalmatian toadflax to compete (Robocker 1974). Revegetation of disturbed areas using competitive grass species has been successful in reducing and preventing the establishment of Dalmatian toadflax seedlings. Robocker (1974) studied the effects of herbicides, fertilizer, and reseeding on Dalmatian toadflax seedling establishment. In that study, crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) was instrumental in reducing establishment of Dalmatian toadflax seedlings.

Biological Control

Biological control efforts currently center around twelve insects which attack *L. genistifolia* ssp. *dalmatica* (Nowierski 1995). The defoliating moth, *Calophasia lunula* (Hufn.), was first released in Montana in 1972 for control of Dalmatian toadflax (Story 1979, Table 2). This insect was found to be established on Dalmatian toadflax in 1989 at a single site approximately 7 miles southeast of Missoula, apparently from releases made during the early 1980s (McDermott et al. 1990). Larvae collected from this site in 1989 and in subsequent years have been used as the rearing stock to produce over 35,000 larvae for release at a number of Dalmatian toadflax and yellow toadflax, *Linaria vulgaris* (L.), Mill, sites in Montana and other states. Five new toadflax insects were approved by the Animal Plant Health Inspection Service - Plant Protection & Quarantine for release against Dalmatian toadflax and yellow toadflax in the U.S. in 1996. These include a stem-galling weevil, *Mecinus janthinus* (Germar), two root boring moths, *Eteobalea intermediella* (Treitschke) and *Eteobalea serratella* (Treitschke), a seed capsule-feeding weevil, *Gymnetron antirrhini* (Payk.) (Dalmatian

toadflax-adapted strain from Yugoslavia), and a root-galling weevil, *Gymnetron linariae* (Payk.). All five of these insects were released in the field against Dalmatian or yellow toadflax in Montana in 1996. Three additional insects are currently being screened by the International Institute of Biological Control in Delémont, Switzerland. These include two stem-galling weevils, *Gymnetron hispidum* (Payk.) and *Gymnetron thapsicola* (Germar), and a Dalmatian toadflax-adapted strain of the seed capsule-feeding weevil, *Gymnetron netum* (Germar)(Table 3).

Two seed capsule-feeding weevils, *G. antirrhini* and *G. netum,* and one ovary-feeding beetle, *Brachypterolus pulicarius* (L.) (Coleoptera: Nitidulidae), were accidentally introduced into North America during the early 1900's (Table 4). All three of these insects attack Dalmatian and yellow toadflax (Smith 1959, Harris 1961, McClay 1992). *Brachypterolus pulicarius* and *G. antirrhini* reportedly are responsible for the decline in the spread of yellow toadflax in Canada (Harris 1961). Harris (1961) reported that *B. pulicarius* reduced the number of yellow toadflax seeds from 5,584 to 305 seeds per flower stem - a seed reduction of over 90%. In research conducted by McClay (1992), *B. pulicarius* reduced the seed production of yellow toadflax by about 74%. *Brachypterolus pulicarius* also feeds on larger more robust Dalmatian toadflax plants (Smith 1959). In Canada, *B. pulicarius* has been found feeding on Dalmatian toadflax stands in the East Kootenay region of British Columbia (Harris 1988, Nowierski 1995). The effects of *Brachypterolus pulicarius* feeding on Dalmatian toadflax have not been documented.

History and Distribution of *Brachypterolus pulicarius*

Brachypterolus pulicarius is a small, black beetle found throughout Europe (Hervey 1927). Linnaeus first described this beetle in 1758 as *Dermestis pulicarius*. Later in 1788 he changed the generic name to *Silpha*. A complete list of the synonymy of *B. pulicarius* is given by Grouvelle, 1913 (Hervey 1927). *Brachypterolus pulicarius* is most commonly associated with feeding in the blossoms of yellow toadflax (Hervey 1927, Harris 1988). The beetle also feeds on other members of the genus including *L. genistifolia* ssp. *dalmatica*, *L. supina* (L.) Hill, and *L. striata* (Scheele) Pennell (Hervey 1927, Harris 1988, Nowierski 1995). *Galium mollugo* L., and *Spiraea ulmaria* Greene also have been reported as food sources or hosts for this nitidulid (Hervey 1927).

Brachypterolus pulicarius was first recorded in the United States in 1919 in the state of New York (Hervey 1927). Interest in this beetle was sparked in the 1920's because of reports of *B. pulicarius* feeding within strawberry blossoms in the Northwest region of the U.S. (Hervey 1927). Adult beetles have been observed on the blossoms of strawberry, dandelion, wild mustard, clover, apple, and dogwood. They apparently do little damage to these plants and migrate to the *Linaria* species as soon as the stems develop (Hervey 1927). By 1953, *B. pulicarius* was found throughout southern Saskatchewan in Canada, presumably following the rapid increase in yellow toadflax infestations in that province a decade earlier (Harris 1961). Currently *B. pulicarius* is widely distributed throughout southern Canada, and the northwestern

United States. *Brachypterolus pulicarius* is commonly found on yellow toadflax and less frequently on Dalmatian toadflax infestations in these areas, including Montana (McClay 1992, Nowierski 1995). In Montana, infestations of Dalmatian toadflax near Lodge Grass, Emigrant, Townsend, Butte, Helena, Radersburg, Missoula, and Moiese were sampled with a sweep net for adult beetles during the 1992 and 1993 field season. *Brachypterolus pulicarius* were found in small numbers on all Dalmatian toadflax sites visited (2-5 beetles per 100 sweeps) (Grubb, Nowierski unpub. data).

Morphological Description of *Brachypterolus pulicarius*

Hervey (1927) provided the following detailed description of the life stages of *B. pulicarius*. The adult beetles are 2.2-2.6 mm long and 1.0-1.2 mm wide, black, partly shiny above, oval, strongly convex, and covered with sparse brown hairs (Figure 2). The legs and antenna are rufous, with the first segment of the antenna and the posterior legs darker than the remainder. The dorsal surface of the body is deeply and thickly punctate, with the punctures on the head and dorsal surfaces of abdominal segments somewhat finer. The posterior sides of scutellum are smooth, shiny, and impunctate. The head is about half as wide as the thorax. The antennae are sub-capitate and club elongate. The thorax is convex and about two-thirds wider than long. The elytra are about one-third longer than the thorax, are rounded and have separated apices. On the female, two abdominal segments are exposed dorsally, with three being exposed on the male. The middle and posterior legs are flattened with the tibiae dilated

at tip and crowned with a row of equal spines. The outer margin of tibiae on both the anterior and middle legs contain a row of spinules.

Eggs are about three fifths of a millimeter long and white in color, turning yellow just before hatching. The larvae are about 5.5 mm long when full grown, strongly convex above, and flattened below. The mandibles, head, and thorax are brownish in color with a light median stripe down the prothorax. The remainder of the body is pale yellow. The larvae have ten abdominal segments, with the tenth being greatly reduced. The head is heart-shaped and somewhat broader than long.

The pupae are 2.8 mm long and 2.0 mm wide and yellow in color. The body is sparsely covered with long brownish hairs which are thickest on the head and last abdominal segment. The legs and wing pads are closely appressed to the body. The tarsi are distinct, but do not show segmentation. The head is drawn into the thorax, and the eyes and mouthparts are pressed close to the body. The labrum and mandibles are distinct with the latter being chitinized. The antennae are club shaped, but not segmented. The dorsal surface of the thoracic segments have four pairs of spines projecting towards the head. Each of the abdominal segments has a pair of spines.

Life History of *Brachypterolus pulicarius*

Adult *B. pulicarius* emerge in the spring (May-June) and feed on the terminal shoots of young toadflax stems causing stooling (Harris 1961, McClay 1992). Mating occurs in June and the females lay their eggs in the flower buds under the corolla (Hervey 1927, Harris 1961). Usually one, but up to three eggs are laid per flower bud

(Hervey 1927). The larvae feed mainly upon the anthers and ovaries in the developing buds and flowers. Older larvae feed to some extent on the maturing seeds within the developing seed capsule (Hervey 1927, Harris 1961, Darwent et al. 1975). The larvae are very mobile and can destroy ovaries in many flowers over the course of their development (Harris 1961). The adult beetles continue to feed in the flowers and terminal shoots of Dalmatian toadflax throughout the summer. Adults die off from mid-August to mid-September depending on climatic conditions (Harris 1961). Larvae feed throughout the summer and pupate in early August to early September. The pupae overwinter about 5 cm deep in the soil near the base of the plant (Hervey 1927, Harris 1961). Hervey (1927) and Harris (1961) claim that adult beetles found feeding in late summer and fall are from a second generation. It is not known whether these adults overwinter.

Objectives of Study

The overall goal of this study was to determine the effects of *B. pulicarius* feeding on the growth and reproduction of Dalmatian toadflax. The specific objectives were to:

(1) Determine the hand pollination technique that would produce maximum fertilization of Dalmatian toadflax flowers.

(2) Evaluate the impact of *B. pulicarius* on seed production of Dalmatian toadflax.

(3) Evaluate the impact of *B. pulicarius* on growth characteristics of Dalmatian toadflax.

(4) Assess strain differences between populations of *B. pulicarius* collected from Dalmatian versus yellow toadflax plants using isozyme analysis.

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CHAPTER 2

HAND POLLINATION OF DALMATIAN TOADFLAX *Linaria genistifolia* ssp. *dalmatica* (L.) MAIRE and PETITMENGIN (SCROPHULARIACEAE)

Introduction

Dalmatian toadflax, *Linaria genistifolia* ssp. *dalmatica* (L.), Maire and Petitmengin (Scrophulariaceae), is a short-lived perennial plant native to the northern Mediterranean region of Europe. This plant is thought to be an escaped ornamental brought to North America in the late 1800's (Alex 1962). It thrives in cool semiarid climates with coarse textured soils (Robocker 1970, 1974). Since its introduction to North America, Dalmatian toadflax has spread to infest much of the northern and western United States, and southwestern Canada (Robocker 1974). In the state of Montana, over 150,000 acres of rangelands are infested by Dalmatian toadflax (Lajeunesse et.al. 1993). Dalmatian toadflax displaces native plant communities and reduces forage for livestock and wildlife (Robocker 1974, Saner 1991). Once established, this weed can affect the composition of the plant community by competing intensively for soil moisture (Robocker 1974, Saner 1991).

Several insects that affect the sexual reproduction of yellow and Dalmatian toadflax are being investigated for use as biological control agents (Nowierski 1995). Two of these insects, *Gymnetron antirrhini* (Payk.) and *Gymnetron netum* (Germar), form galls within toadflax seed capsules, and a third insect, *Brachypterolus pulicarius* (L.) primarily causes feeding damage to the ovaries and developing seeds (Hervey

1927, Smith 1959). McClay (1992) used hand pollination of yellow toadflax flowers to study the effects of *B. pulicarius* on the flowering and seed production of yellow toadflax. However, the timing and frequency of hand pollination may affect the results and conclusions of studies on flower and seed-feeding insects. Hand pollination methods can alter pollen germination rates and paternity of the resulting seeds, seed production, and total yield in many plant species (Patterson 1989, Mitchell and Marshell 1995, Brookfield et al. 1996). In a study on the effects of *B. pulicarius* on Dalmatian toadflax, Grubb (unpub. data) found differences in seed production in control pots between similar studies that used different hand pollination schemes. Mitchell and Marshell (1995) suggested that experiments involving hand pollination should employ pollination methods that try to mimic the natural arrival of pollen in nature.

Honey bees and bumblebees are among the most important pollinators in many plant communities. Such types of bees are known to exhibit flower consistency; if foraging success is high, bees will fly repeatedly and directly among individuals of that species, and provide efficient pollination (Thomson 1986, Goulson 1994). Bumblebees and halictid bees reportedly are the main pollinators of both yellow and Dalmatian toadflax (Alex 1962, Arnold 1982).

In order to assess the efficacy of biocontrol agents that affect sexual reproduction of Dalmatian toadflax in inclusion studies, it was important to develop hand pollination protocols which produced consistent and predictable fertilization.

Hence, the objective of this study was to determine the hand pollination scheme which produced the highest percent fertilization of Dalmatian toadflax flowers.

Materials and Methods

Study location

The study was conducted in greenhouse facilities at the Montana State University Plant Growth Center, Bozeman, MT. The greenhouse temperatures ranged from 20 to 26° C, with a 14 hour light, 10 hour dark photophase.

Plant materials and pollen collection

Plants for this study were grown from seeds collected near Radersburg, MT in August 1990. Seeds were stored in vials at room temperature. Plants used in this experiment were sown on 3 Jan. 1992. Seedlings (5 cm in height) were transplanted into 15 cm diameter plastic pots with a 20 cm soil depth. Pots were filled with a pasteurized soil mixture of one-half Farland silt loam (fine-silty, mixed Typic Argiboroll), and one-half sand. Plants were watered to pot capacity every other day. Pollen was collected off Dalmatian plants not used in the study and placed into a 10 cm petri dish. It was then applied to Dalmatian toadflax flowers in the study with a fine artist's paintbrush on the day it was collected.

Procedures

The experiment was initiated on 12 Apr. 1992. Thirty-eight mature Dalmatian toadflax plants were trimmed to two flowering stems and the dead foliage

removed. Plants were arranged in a randomized-complete-block design, with nineteen treatments and two replications per treatment. For each treatment, all flowers on a single plant were hand-pollinated on a treatment day(s) corresponding to the day the corolla opened. Hand pollination treatments used in this study are listed in Table 5.

After hand pollination, each flower was individually tagged and marked with: treatment, bud number, date flower opened, day(s) pollinated, and date flower dropped. The presence of seed capsules was recorded, and the capsules were left to mature. Height, depth, and width of the seed capsules were measured. Volume of seed capsules was determined using the equation for the volume of an ellipse rotated about its major axis ($4/3*\pi$[width*height*depth]; Selby 1969). This shape approximates that of a Dalmatian toadflax seed capsule. Analysis of variance (ANOVA, SAS Institute, 1991) was used to determine the effects of treatments on the percent pollination and volume of seed capsules. Percent pollination was calculated by dividing the total number of seed capsules produced per plant by the total number of flowers pollinated per plant. Percent data was transformed using an arc sine transformation for the analysis of variance and mean separation procedures. Mean separations were calculated using the Student Newman-Keuls mean comparison test (ANOVA, SAS Institute, 1991). Data was transformed back into percentages for discussion.

Results

Seed Capsule Production

Significant differences in percent seed capsules produced were found for different hand pollination treatments (Table 6, $P < 0.001$). Hand pollination schemes that included pollination on the day the corolla opened and/or the four days following (treatments 1-5,11-13,16 and 18) produced the highest percent of capsules (Table 7). Capsule production ranged from around 72 to 100 percent in these treatments. Flowers pollinated a single time on day eight or nine (treatments 9,10), or on both days eight and nine (treatment 15), produced the lowest percent of seed capsules. These treatments produced similar percent capsules as unpollinated flowers (treatment 19). Capsule production on all other treatments ranged from around 33 to 60 percent.

Seed Capsule Volume

Significant differences in the volume of seed capsules (Table 8, $P < 0.001$) were observed among the various treatments. Flowers pollinated on both days eight and nine (treatment 15) produced the largest seed capsule volume (Table 9). Flowers pollinated a single time on days three (treatment 4) or five (treatment 6) produced the smallest capsule volumes. Capsule volume resulting from a single pollination on day two (treatment 3) was about 20 mm^3, which was similar to flowers pollinated a single time on day four (treatment 5). All other treatments were similar producing capsule volumes ranging from 29 to 38 mm^3.

Discussion

This results of this study suggest that hand pollination can provide effective and consistent fertilization of Dalmatian toadflax. Pollination success is highest when flowers are hand pollinated within the first four days of bloom. Similarly, in a study on the pollination, predation and seed set of yellow toadflax, Arnold (1982) found that the mean lifespan of flowers for successful cross-pollination by hand was 3.9 days. Multiple hand pollination of Dalmatian toadflax flowers consistently produced large volume capsules.

Halictid bees must visit yellow toadflax flowers at least twice to ensure that enough out-cross pollen is available to fertilize all to ovules in an ovary (Arnold 1882). The "pollen population effect" theory proposed by Brewbaker and Majumder (1961) states that pollen germination increases with the density or number of pollen grains on the stigma. An increase in pollen germination may have an affect on the number of seeds produced. Multiple hand pollination of Dalmatian toadflax flowers could ensure that there is enough pollen to fertilize all the ovules in an ovary and also may increase pollen germination rate. Based on seed capsule volume and the percent of capsules produced, the highest fertilization success of Dalmatian toadflax grown in the greenhouse or cage studies occurs when flowers are hand pollinated twice within the first four days of bloom. Interestingly, repeated pollination does not appear to compensate for late pollination.

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CHAPTER 3

EFFECTS OF *Brachypterolus pulicarius* (L.) (COLEOPTERA: NITIDULIDAE) ON GROWTH AND SEED PRODUCTION OF DALMATIAN TOADFLAX *Linaria genistifolia* ssp. *dalmatica* (L.) MAIRE AND PETITMENGIN (SCROPHULARIACEAE)

Introduction

Over the past century, the loss of native grasslands in North America has largely been associated with the invasion of aggressive, non-native (noxious) weeds (Roche and Talbott 1986). One such noxious weed is Dalmatian toadflax, *Linaria genistifolia* ssp. *dalmatica*, which is of Eurasian origin. Dalmatian toadflax is a short-lived perennial introduced to the United States as an ornamental plant in 1894 (Alex 1962). The weed has infested rangelands in the western and northeastern United States and southern Canada (Robocker 1974, Figure 1). This species can be very competitive in disturbed areas and difficult to manage (Lajeunesse et al. 1993, Nowierski 1995). Mature Dalmatian toadflax plants compete intensively for soil moisture and can displace native vegetation (Robocker 1974). Although both livestock and wildlife have been reported to consume the floral stems of Dalmatian toadflax, the plant is not considered to be a high-value forage (Lange 1958, Reed and Hughes 1970). Polunin (1969), and Reed and Hughes (1970) reported that Dalmatian toadflax, if ingested in large quantities, can be toxic to livestock. Losses in rangeland production can be

sustained through direct competition and reduced quality and/or palatability of the forage as the density of toadflax increases (Nowierski 1995).

Brachypterolus pulicarius (L.) (Coleoptera: Nitidulidae) is a small, oval, convex ovary-feeding nitidulid, 2.2-2.6 mm in length, and 1.0-1.2 mm wide (Hervey 1927). Adult beetles feed on new growth at the tips of the stems and on the axillary buds at the base of the leaves as well as within the flowers upon the anthers and ovaries (Harris 1961, Darwent et al.1975, McClay 1992). Adult beetles generally feed from late-May to mid-August (Harris 1961, McClay 1992). One egg per bud is usually laid in mid-July under the corolla of unopened buds (Hervey 1927). Larvae emerge three to five days later and feed mainly on the anthers, ovaries, and to some extent the maturing seeds (Hervey 1927, Harris 1961). Larvae are mobile and destroy ovaries in many flowers over the course of their development (Hervey 1927, McClay 1992). Pupae are formed in late summer and overwinter in the soil near the base of the plant (Hervey 1927).

Brachypterolus pulicarius was accidentally introduced into North America and was discovered on this continent around 1919. This beetle feeds on both yellow toadflax, *Linaria vulgaris* (L.) Mill, and Dalmatian toadflax (Hervey 1927, Smith 1959). *Brachypterolus pulicarius*, in conjunction with a seed-feeding weevil, *Gymnetron antirrhini* (Payk.) (Coleoptera: Curculionidae) have been attributed to the reduced rate in the spread of yellow toadflax in Canada (Harris 1961).

Although Harris (1961) and McClay (1992) reported that *B. pulicarius* reduced seed production of yellow toadflax by 74 to 90%, the impacts of this insect on

Dalmatian toadflax have not been documented. The objective of this study was to evaluate the effects of ***B. pulicarius*** on the growth and seed production of Dalmatian toadflax. We hypothesized that feeding damage by ***B. pulicarius*** will cause stooling of the plant and reduce the seed production of Dalmatian toadflax.

Materials and Methods

Study locations

The study consisted of two greenhouse experiments and one field experiment. Greenhouse experiments were conducted at the Montana State University-Bozeman Plant Growth Center. The greenhouse was set at 23° C, with a 14 light, 10 hour dark photophase. Minimum and maximum greenhouse temperatures ranged from 20-26° C throughout the study. The field experiment was conducted at a Montana State University research site located on the Bozeman campus with an elevation of 1330 m and zero slope (45° 36' 26" N, 111° 5' 36" W). Soils were a sandy loam. Annual precipitation at the site ranges from 381 to 483 mm, and the frost-free period ranges from 90 to 110 days. Total precipitation during the duration of the 1993 field study was 235 mm.

Plant materials and insect collection

Plants for this study were grown from seed collected near Radersburg, MT in August of 1990. Seeds were stored in vials at room temperature. Plants used in the 1992 greenhouse experiment were sown in the spring of 1991, 3 Jan. 1992, and 26

Feb. 1992. For plants used in the 1993 greenhouse and field experiments, seeds were sown 13 Apr. 1992, 19 Dec. 1992, and 2 Mar. 1993. Seedlings (5 cm in height) were transplanted into 15 cm diameter plastic pots with a 20 cm soil depth. Pots were filled with a pasteurized soil mixture of one-half Farland silt loam (fine-silty, mixed Typic Argiboroll), and one-half sand. Plants were watered to pot capacity every other day, and fertilized monthly with Peters® liquid 20-20-20 fertilizer. Plants were grown for three, six, and 12 months. Plants used in the field experiment were transplanted from pots to the field location on 21 May 1993. Adult *B. pulicarius* used in this study were collected from Dalmatian toadflax infestations near Kamloops, British Columbia, Canada on 30, 31 May 1993, and 1, 2 Jun. 1993.

Procedures

The experiments were initiated on 1 Jun. 1992 (greenhouse) and 6 Jun. 1993 (greenhouse, field) by releasing 10 un-sexed *B. pulicarius* on individual plants of each age (three, six, and 12 months after sowing). A comparative set of plants from each age remained untreated. Experiments were replicated eight times in a split-plot design with age as wholeplots and presence or absence of *B. pulicarius* as subplots (2 - *B. pulicarius* treatments, 3 - ages, 8 - replications). In 1992, as flowers opened they were hand-pollinated twice weekly. Flowers were pollinated every other day in both 1993 experiments. Pollen was collected from Dalmatian toadflax plants not used in the study and applied with a fine artists paint brush. Individual plants were covered with a nylon sleeve cage supported by a wire and/or PVC frame about 72 cm tall. Experiments

were terminated after all adult beetles had died, and the larval *B. pulicarius* had pupated. The 1992 and 1993 greenhouse experiments were terminated on September 14, 1992 and Sept 4, 1993, respectively. The field experiment was terminated on September 12, 1993.

Sampling

All sampling occurred every two weeks. In all experiments, overall plant height (cm) was recorded. The number of primary and secondary stems and branches of Dalmatian toadflax were counted and measured for length (cm). In the greenhouse experiments, the number of flower buds, flowers, and seed capsules were also counted. Mature seed capsules were removed prior to dehiscence. Seeds were removed from capsules, carefully hand-separated from chaff, and weighed and counted. Length, width, and height of each capsule was measured. Capsule volume was determined using the equation for an ellipse rotated about its major axis ($4/3*\pi$[width*height*depth]; Selby 1969). The elliptical volume approximated capsule shape. In the 1993 field experiment, the number of Dalmatian toadflax plants that flowered were inadequate for analysis.

Statistical Analysis

Each experiment was analyzed separately using split-plot analysis of variance (SAS Institute, 1991). Age was tested using the pooled mean square of block x age as the error term. Treatment (presence or absence of *B. pulicarius*) and age x treatment

were included in the subplot analysis and were tested using the pooled mean square of block x age x treatment as the error term. Error bars represent $\pm$ 1SE.

The direct and indirect effects of each variable on seed production and seed weight were estimated using a path coefficient model (Li 1975, Wright 1977, Jordon 1989). Each arrow in the path diagram indicates a hypothesized effect of one growth variable on another. We hypothesized that all growth variables had both direct and indirect effects on seed production and seed weight. Path coefficients were used to estimate hypothesized causal effects. These were partial regression coefficients indicating the effect of one growth variable on a second when other variables that affected both variables were statistically held constant.

Results

Impacts of B. pulicarius on Dalmatian toadflax variables

Height: In 1992, the effects of *B. pulicarius* on Dalmatian toadflax height were dependent on plant age at the time of insect release (Table 10). Height of plants grown for three months prior to *B. pulicarius* release were reduced by about 23 cm, while height of plants grown six months prior to insect release were reduced by only 7.4 cm (Figure 3). Height of plants grown for 12 months prior to insect release were unaffected by *B. pulicarius*. In 1993, the effects of *B. pulicarius* on Dalmatian toadflax height was not dependent upon plant age at the time of insect release (Table 10). *Brachypterolus pulicarius* reduced the height of Dalmatian toadflax by 7.5 cm and

13.3 cm in the 1993 greenhouse and field studies, respectively (Table 11). In that year, height of plants increased as the growing period prior to *B. pulicarius* release was longer.

Flowering stems: The number of flowering stems of Dalmatian toadflax were not significantly affected in the 1992 greenhouse and the 1993 greenhouse and field studies by *B. pulicarius* feeding (Table 10). In the 1993 greenhouse experiment, the mean number of flowering stems per plant increased as the growing period prior to insect release was lengthened (3 mo.= 1.2, 6 mo. = 4.8, 12 mo. = 6.2; SE(model) = 0.6).

Primary and secondary branches: Feeding by *B. pulicarius* increased the number of Dalmatian toadflax primary branches by 52 to 77% (Tables 10 and 12). In the greenhouse, *B. pulicarius* increased the number of secondary branches from 1.1 to 21.1 (SE(model)) = 6.5) in 1993 with no apparent effect from plant age. However, in the 1992 greenhouse and 1993 field studies, the effects of *B. pulicarius* on secondary branching were dependent on plant age at the time of insect release (Figure 4). In both cases, the greatest number of secondary branches were produced on plants which were six months old at the time of *B. pulicarius* release. In 1992, plants receiving *B. pulicarius* after three and twelve months were similar and produced fewer secondary branches than six month old plants. Twelve month old plants produced significantly more branches than three month old plants in the 1993 field study. Those plants without *B. pulicarius* produced the lowest amount of secondary branches in both years.

Flower buds and flowers: *B. pulicarius* feeding decreased the number of Dalmatian toadflax flower buds by 15% in 1992 (Tables 10 and 13). Buds were unaffected in 1993. Insect feeding decreased the number of flowers by 44 and 49% in

1992 and 1993, respectively. In 1992, plants grown for three months prior to *B. pulicarius* release had fewer flower buds compared to plants grown six or twelve months prior to insect release. The number of flower buds were similar on six and twelve month old plants. Number of flower buds in 1993 increased as the growing period prior to insect release was lengthened. In 1992, the number of flowers increased as the growing period prior to the release of *B. pulicarius* was greater. Plants grown three to six months before insect release had fewer flowers than on plants grown for 12 months in 1993. In that year, the number of flowers were similar on three and six month old plants.

Seed capsules and seeds: Effects of *B. pulicarius* feeding on seed capsules and seeds were dependent on the age of the plants at the time of insect release (Table 10). In all cases, the greatest number of seed capsules and seeds were produced on the oldest plants in the absence of *B. pulicarius*. On plants receiving insects after twelve months, seed capsules were reduced by 13.8 and 71.3 and seed production by 1462 (60%) and 5646 (69%) in 1992 and 1993, respectively (Figures 5 and 6). On plants receiving insects after six months of growth, *B. pulicarius* reduced capsules by 4.3 in 1992 but did not significantly affect them in 1993. On those plants, *B. pulicarius* reduced seed production by 677 (43%) and 1267 (84%) in 1992 and 1993, respectively. *B. pulicarius* reduced capsules on the youngest plants in 1992 by 6.7 but did not significantly affect them in 1993. Seed production on plants receiving insects after three months of growth was reduced by 726 (72%) in 1992 and 459 (93%) in 1993.

Seed weight and seed capsule volume: B. pulicarius reduced seed weight in both years by an average of 0.0065 mg (Tables 10 and 14). In the 1993 greenhouse

experiment, seed weight increased as the growing period lengthened (3 mo.= 0.006, 6 mo. = 0.011, 12 mo. = 0.016; SE(model) = 0.002). *B. pulicarius* increased Dalmatian toadflax seed capsule volume in 1992 but decreased capsule volume in 1993.

Relationships among Dalmatian toadflax variables

In the absence of *B. pulicarius*, path analysis indicated that the direct variables which had a significant positive influence on Dalmatian toadflax seed production in both 1992 and 1993 were the number of flowers and seed capsules produced per plant (Figure 7). The influence of flower numbers on Dalmatian toadflax seed production was removed when *B. pulicarius* was present. The influence of seed capsules remained positive, and the value of the path coefficients was increased from 0.65 to 0.78 and 0.30 to 0.98 in 1992 and 1993 respectively in the presence of *B. pulicarius*. In 1992 and 1993, the number of flower buds positively influenced the number of flowers, and the number of flowers positively influenced the number of seed capsules in the absence of *B. pulicarius*. In the presence of *B. pulicarius*, the number of buds positively influenced the number of flowers in both years, but the influence of flower numbers on the number of capsules was removed.

The number of primary and secondary branches was unrelated to seed production in the absence of *B. pulicarius* (Figure 7). In the presence of *B. pulicarius*, the number of primary branches positively influenced seed production, while seed production was negatively influenced by the number of secondary branches.

In 1993, seed capsule volume positively influenced seed weight in the absence of *B. pulicarius* (Figure 7). In the presence of *B. pulicarius*, seed capsule volume was positively related to seed weight in both years. All other relationships in the path analysis were either inconsistent between years and/or did not influence seed production or weight.

Discussion

The results obtained in this study are similar to the observations obtained by Selleck et al. (1957) and Harris (1961) for yellow toadflax in that *B. pulicarius* adults caused stooling of the toadflax plants by feeding on the young stems. In this study, *B. pulicarius* reduced the height and increased primary and secondary branching of Dalmatian toadflax. In one experiment, the effects of insects on height were greater on the youngest plants (3 mo.). In general, the greatest number of secondary branches occurred on moderately aged plants (6 mo.) containing *B. pulicarius*.

Branching effects may have a direct impact on seed production. *B. pulicarius* feed on the early, succulent growth of Dalmatian toadflax (Smith 1959). Initial feeding on the apical growing points of stems stimulated primary branching. Path analysis indicated that this increase in primary branching, in itself, increased seed production. However, continued feeding on the apical points of primary branches caused secondary branching. We speculate that resources ordinarily used for flower and seed production are exhausted during secondary branching. The negative relationship between secondary branching and seed production shown in the path analysis supports this hypothesis.

B. pulicarius reduced the number of Dalmatian toadflax flowers, which removed the positive relationship between flower production and seed capsules and the relationship between flower production and seed production. Both larval and adult stages of *B. pulicarius* feed on the anthers and ovaries in the developing buds and flowers (Hervey 1927, Harris 1961, Darwent et al. 1975). Therefore, flowers may have been present but incapable of developing seed capsules. The relationship between the number of seed capsules and seed production remained positive.

This study showed that *B. pulicarius* reduced seed production between 43 to 93% depending upon the age of the plant at time of release. There may be two primary mechanisms for seed reduction. Secondary branching may exhaust resources prior to flower formation, and *B. pulicarius* may affect the ability of the flowers to produce seed capsules. McClay (1992) reported similar results in that *B. pulicarius* feeding reduced the viable seed production of yellow toadflax by 74.1% or about 841 seeds per plant. McClay attributed this reduction either to inhibition of flowering or to reduced seed set per flower.

Successful weed management will ultimately be based on our understanding of the life history of plant populations and how management strategies impact mechanisms and processes directing population and community dynamics (Sheley and Larson 1996, Maxwell and Sheley 1997). *Brachypterolus pulicarius* affects reproduction allocation, seed production, dispersal, and may reduce the potential for rapid adaptation. Therefore, *B. pulicarius* may prove to be a major component in the integrated weed management of Dalmatian toadflax.

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CHAPTER 4

ISOZYME ANALYSIS OF *Brachypterolus pulicarius* (L.) (COLEOPTERA: NITIDULIDAE) COLLECTED FROM DALMATIAN TOADFLAX *Linaria genistifolia* ssp. *dalmatica* (L.) MAIRE AND PETITMENGIN (SCROPHULARIACEAE) AND YELLOW TOADFLAX *Linaria vulgaris* (L.) MILL (SCROPHULARIACEAE)

Introduction

Dalmatian toadflax, *Linaria genistifolia* ssp. *dalmatica* (L.) Maire and Petitmengin (Scrophulariaceae), is a non-indigenous perennial weed native to the Mediterranean region of Europe and western Asia. It has been used as an ornamental plant since the late 1500's, and was apparently brought to North America around 1894 for these same purposes (Alex 1962). Because of its invasive nature, Dalmatian toadflax has been able to infest drier open areas, rangelands, roadsides, and disturbed sites throughout the western and northeastern United States and southern Canada (Robocker 1974, Figure 1). Although seedling establishment is poor initially, once established, Dalmatian toadflax is highly competitive and difficult to eradicate (Robocker 1974, Nowierski 1995). Mature Dalmatian toadflax plants compete effectively for soil moisture and displace native vegetation (Robocker 1974). Dalmatian toadflax produces low quality forage, and can be toxic if ingested in large quantities (Lange 1958, Polunin 1969, Reed 1970). The weed also reduces species diversity and adversely affects forage production for livestock and wildlife (Saner 1991, Nowierski 1995).

Brachypterolus pulicarius (L.), an ovary feeding beetle, was accidentally introduced into North America around 1919 (Hervey 1927, Harris 1961). The beetle commonly feeds within the flowers upon the anthers and ovaries of yellow toadflax (*Linaria vulgaris*) (L.) Mill (Hervey 1927, Harris 1961). Harris (1961) and McClay (1992) reported that *B. pulicarius* reduced seed production of yellow toadflax by 74 to 90%, respectively. Harris (1961) also attributed the decline in spread of yellow toadflax in Canada to feeding by *B. pulicarius* and a seed capsule feeding weevil, *Gymnetron antirrhini* (Payk.) (Coleoptera: Curculionidae). *Brachypterolus pulicarius* feeds on several other members of the genus *Linaria* including *L. supina* (L.) Hill, *L. striata* (L.) (Scheele) Pennell and Dalmatian toadflax, *L. genistifolia* ssp. *dalmatica* (Hervey 1927, Harris 1988, Nowierski 1995).

Brachypterolus pulicarius is widely distributed on yellow toadflax infestations throughout southern Canada and the northwestern United States. Adult beetles are found less commonly and usually less abundantly on Dalmatian toadflax infestations in these areas (McClay 1992, Nowierski 1995). If *B. pulicarius* is a truly oligophagous insect feeding on both yellow and Dalmatian toadflax, it should show a high degree of demographic exchangeability throughout its distribution performing equally well on either host. However, if biotypes, or host races exist in *B. pulicarius* the demographic exchangeability and performance of this insect may be skewed because of differing preferences or tolerances for each host. This may explain the contrast in abundance of *B. pulicarius* on yellow toadflax versus Dalmatian toadflax in North America.

In a study of the thistle head weevil, *Rhinocullus conicus* (Froelich), Unruh and Goeden (1987) emphasized the importance of correctly matching insect host races with target host plants. Three European host races of *R.conicus* were identified attacking thistle species from several genera including *Carduus, Cirsium, Onopordum,* and *Silybum* (Zwolfer and Preiss 1983). The host races were not discovered until twelve years after the introduction of *R. conicus* into the United States as a biological control agent. This resulted not only in the failure of early efforts to establish *R. conicus* on milk thistle (*Silybum marianum* L.) in California, but also in the undesirable attack on a number of native thistle species (Goeden et al. 1981, Turner et al. 1987). Releasing phytophagous biological control agents in this trial and error fashion can result in a overestimation of an insects potential to control a particular plant pest, while forfeiting time, effort, and resources in the process (Rosen 1978, Bush and Hoy 1983). To help maximize biological control efforts using *B. pulicarius* on Dalmatian toadflax, it is important to determine if strain differences or host races exist between populations of this insect on Dalmatian versus yellow toadflax. In this study, isozyme analysis using starch gel electrophoresis was used to examine genetic differences between populations of *B. pulicarius* collected from Dalmatian toadflax and yellow toadflax.

Materials and Methods

Insect Collections

Nine populations of *B. pulicarius* were used in the electrophoretic analysis. Adult *B. pulicarius* from four of those populations were collected from Dalmatian

toadflax plants near Kamloops, Canada (Table 15). The remaining five populations were collected from yellow toadflax plants at various sites in southwestern Montana. Approximately 150 adult beetles were aspirated from flowering toadflax plants at each site. Aspiration vials containing *B. pulicarius* adults were labeled by collection site, and placed into a 15 gallon Igloo® cooler chilled with frozen blue ice for transport back to the laboratory at Montana State University. Upon arrival at the laboratory, 100 live adults from each collection site were individually placed into 8 dram glass vials and frozen at -80° C for subsequent isozyme analysis.

Electrophoretic Techniques

Electrophoresis was performed on horizontal starch gels using 12% hydrolyzed potato starch (#S-4051; Sigma, St. Louis, MO), and 500 ml gel buffer from St. Leger et al. (1992). Individual beetles were ground in four drops of extraction buffer (0.5 M TRIS-HCl, pH 6.8), with the supernatant being wicked into three filter paper wicks 5 mm by 10 mm) cut from Whatman #4 filter paper. This allowed each individual to be run on three different gels simultaneously. The power used for each gel was 17.0 V/cm for a modified lithium buffer (LiOH) system from Vawter and Brussard (1975), 10 V/cm with tris-citrate buffer pH 8.0 (TC 8.0), and 13.5 V/cm for a tris-malate buffer pH 7.4 (TM 7.4) both from Pasteur et al. (1988). Each gel was run for approximately six hours, then sliced horizontally into six slices, each 1.6 mm in thickness. Each slice was subsequently stained for 1 of 17 enzyme systems. After screening the 17 enzyme systems on each of the three buffers, AAT, IDH, LDH, SDH,

and GAPDH were run on LiOH gels; MDH, ME, SOD, MPI, XDH, and PEP were run on TC 8.0 gels; and AK, G6PDH, GPI, PGM, HK, and EST were run on TM 7.4 gels. Enzyme systems, enzyme commission numbers, abbreviations, and number of scorable loci for the 17 enzymes are listed in Table 16. Specific buffer systems and staining protocols are listed in Tables 17 and 18 respectively. Recipes for buffers used in the staining protocols are included in Table 19.

Statistical Analysis

The allelic frequency data from the nine *B. pulicarius* populations (Table 20) were used to calculate Nei's genetic identity and Nei's genetic distance matrices (Table 21, Nei 1972). Nei's genetic distance (D_{ij}) is expressed as follows (Nei 1972):

$$D_{ij} = -\ln I_{ij} \qquad \text{Eq.[1]}$$

$$\text{where, } I_{ij} = \left\{ \frac{\Sigma_k \mid x_{ki}\, x_{kj} \mid}{(\Sigma_k x^2_{ki} \Sigma_k x^2_{kj})^{1/2}} \right\} \qquad \text{Eq.[2]}$$

I_{ij} is called the genetic identity between populations *I* and *j*, which is a ratio of the proportions of loci that are alike within and between populations (Weir 1990). These calculations were performed for all pairwise combinations using the statistical software package NTSYS-PC (Rohlf 1993). Nei's genetic distance was clustered by the un-weighted pair-group method using an arithmetic average to produce a dendrogram. Because the outcome of cluster analysis may be influenced by the particular algorithm chosen (Manly 1994), correspondence analysis was also conducted to provide a more

objective approach for determining the genetic relationships of beetle populations. This was accomplished using a correspondence analysis ordination procedure of the allelic frequencies using the statistical software package CANOCO (Ter Braak 1988).

Results

Allelic frequencies for 27 loci of *B. pulicarius* are presented in Table 20. Patterns of genetic variation among the nine populations of *B. pulicarius* resulting from the cluster analysis of allelic frequencies are presented in a dendrogram (Figure 8). The four populations of *B. pulicarius* collected from Dalmatian toadflax form one distinct grouping in the cluster analysis, while the populations collected from yellow toadflax form a second distinct grouping at a Nei's genetic distance of 0.0118 (Table 21). The one exception is a population of *B. pulicarius* collected from yellow toadflax northwest of Radersburg MT (YT-5), which grouped with the Dalmatian toadflax populations (Figure 8). Surprisingly, this population showed the smallest Nei's genetic distance (0.0010) to a *B. pulicarius* population collected from Dalmatian toadflax in Kamloops, British Columbia, Canada (DT-2) (Table 21, Figure 8). Nei's genetic distances between populations of *B. pulicarius* collected from Dalmatian toadflax ranged from 0.0019 - 0.0129 (Table 21).

Among the populations of *B. pulicarius* from yellow toadflax which clustered together, beetle populations collected near Ovando, MT (YT-2) and 14-mile road near Radersburg, MT (YT-3) were most similar to one another with a Nei's genetic distance of 0.0013 (Table 21). Nei's genetic distances between populations of *B. pulicarius*

collected from yellow toadflax ranged from 0.0013 - 0.0139 (Table 21). These results are corroborated by the correspondence analysis which shows populations DT-1, DT-2, DT-3, DT-4, and YT-5 forming one discrete cluster, and populations YT-1, YT-2, YT-3, and YT-4 forming another cluster (Figure 9).

Discussion

Although no fixed allelic differences with host plant preferences were observed with *B. pulicarius*, significant allelic frequency differences were observed between beetle populations associated with different host plants. *Brachypterolus pulicarius* populations tended to group by host plant with the exception of one beetle population collected from yellow toadflax along South Crow Creek near Radersburg, MT (YT-5). The YT-5 population was most closely associated with a beetle population collected near the Kamloops Community College, Kamloops, Canada (DT-2) with a genetic identity of 0.9990 (Table 21). At the South Crow Creek location both yellow and Dalmatian toadflax infestations were present which may explain why this population was more genetically similar to beetle populations taken from Dalmatian toadflax. McDermott et al. (submitted) found similar results in an isozyme analysis study examining potential host races of *G. antirrhini* and *Gymnetron netum* Germar (Coleoptera: Curculionidae). Significant allelic frequency differences were observed between *Gymnetron* populations associated with different host plants. The one exception was a population of *Gymnetron antirrhini* collected from Dalmatian toadflax near Livingston, MT which were more closely related genetically and

morphometrically to European *G. antirrhini* populations collected from yellow toadflax. Both Dalmatian and yellow toadflax are found at the Livingston collection site.

Measurable levels of genetic variability between *B. pulicarius* populations associated with different host plants were found using this isozyme technique on nine populations of *B. pulicarius* suggesting that two host races may exist in *B. pulicarius*. Increasing the number of populations taken from each host plant or using genetic analysis techniques such as RAPID-PCR may help clarify if host races do exist in *B. pulicarius*.

Results of this study suggest that particular strains of *B. pulicarius* may be better adapted to particular host plants. Such information is of critical importance to weed managers since one of the main objectives of biological control of weeds is to match natural enemies with the correct host plant. This type of information is essential in developing biological control strategies and in assessing the efficacy and/or feasibility of biological control agents (Berlocher 1979, Gonzales et al. 1979, Bush and Hoy 1983, Goeden et al. 1985, McDermott et al. submitted). Releasing strains or host races of a particular natural enemy on host plants that it is not adapted to may result in poor establishment, reduced impacts, and/or undesirable environmental and ecological impacts on nontarget species (Rosen 1978, Bush and Hoy 1983, Lockwood 1993, Louda et al. 1997, Strong 1997).

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APPENDIX A

TABLES 1-21

Table 1. Ideal characteristics of weeds[1].

1)	Germination requirements fulfilled in many environments.
2)	Discontinuous germination (internally controlled) and longevity of seed.
3)	Rapid growth and establishment.
4)	Continuous seed production throughout the growing season.
5)	Self-compatibility but not complete autogamy or apomixy.
6)	Cross-pollination, when it occurs, by unspecialized visitors or wind,
7)	Very high seed output in favorable environmental conditions.
8)	Some seed production in a wide range of environmental conditions, tolerance and plasticity.
9)	Adaptations for short and long distance seed dispersal.
10)	Ability to compete interspecifically by special means (rosette, choking growth, allelochemicals).
11)	If a perennial, vigorous vegetative reproduction or regeneration from fragments and/or brittleness so as not to be drawn from the ground easily

[1] (Baker 1974, Radosevich and Holt 1984, Ross and Lembi 1985, Saner 1991).

Table 2. Biological control insects screened, approved, and released in North America[1].

A) *Calophasia Lunula* (Hufn.) (Lepidoptera: Noctuidae)	Defoliating moth first released in the United States in 1968.
B) *Macinus janthinus* (Germar) (Coleoptera: Curculionidae)	Stem-galling weevil first released in Montana in 1996.
C) *Eteobelea intermediella* (Treitschke) (Lepidoptera: Cosmopterigidae)	Root-boring moth first released in Montana in 1996.
D) *Eteobelea serratella* (Treitschke) (Lepidoptera: Cosmopterigidae)	Root-boring moth first released in Montana in 1996.
E) *Gymnetron antirrhini* (Payk.) (Coleoptera: Curculionidae)	Dalmatian toadflax strain was first released in Montana in 1996.
F) *Gymnetron linariae* (Payk.) (Coleoptera: Curculionidae)	Root-galling weevil first released in Montana in 1996.

[1](Hervey 1927, Smith 1959, Nowierski 1995).

Table 3. Biological control insects currently undergoing screening for release in North America[1]

A) *Gymnetron hispidum* (Payk.) (Coleoptera: Curculionidae)	Stem-galling weevil.
B) *Gymnetron thapsicola* (Germar) (Coleoptera: Curculionidae)	Stem-galling weevil.
C) *Gymnetron netum* (Germar) (Coleoptera: Curculionidae)	Seed capsule-feeding weevil. (Dalmatian toadflax strain)

[1](Hervey 1927, Smith 1959, Nowierski 1995).

Table 4. Biological control insects accidentally introduced to North America[1]

A) *Gymnetron antirrhini* (Payk.) (Coleoptera: Curculionidae)	Seed capsule-feeding weevil first reported in North America in 1909.
B) *Gymnetron netum* (Germar) (Coleoptera: Curculionidae)	Seed capsule-feeding weevil reported North America around 1937.
C) *Brachypterolus pulicarius* (L.) (Coleoptera: Nitidulidae)	Ovary-feeding beetle first reported in North America in 1919.

[1](Hervey 1927, Smith 1959, Nowierski 1995).

Table 5. Hand pollination treatments of Dalmatian toadflax flowers.

Treatment	Hand pollination
1	Flowers pollinated on the day the corolla opened (day 0).
2	Flowers pollinated on the 1st day after the corolla opened (day 1).
3	Flowers pollinated on the 2nd day after the corolla opened (day 2).
4	Flowers pollinated on the 3rd day after the corolla opened (day 3).
5	Flowers pollinated on the 4th day after the corolla opened (day 4).
6	Flowers pollinated on the 5th day after the corolla opened (day 5).
7	Flowers pollinated on the 6th day after the corolla opened (day 6).
8	Flowers pollinated on the 7th day after the corolla opened (day 7).
9	Flowers pollinated on the 8th day after the corolla opened (day 8).
10	Flowers pollinated on the 9th day after the corolla opened (day 9).
11	Flowers pollinated on days 0 and 1.
12	Flowers pollinated on days 2 and 3.
13	Flowers pollinated on days 4 and 5.
14	Flowers pollinated on days 6 and 7.
15	Flowers pollinated on days 8 and 9.
16	Flowers pollinated on days 0 through 4.
17	Flowers pollinated on days 5 through 9.
18	Flowers pollinated on days 0 through 9.
19	Control (Flowers not pollinated).

Table 6. Results of analysis of variance of percent seed capsules produced by hand pollination treatment. Refer to Table 5 for hand pollination treatments.

Source	DF	S.S.	M.S.	F-Value	P-Value
Grand Mean(38) =61.6					
Total (adj. for mean)	37	31561.			
Treatment	18	30650.	1702.8	35.5	0.0001
Residual	19	910.7	47.9		

Table 7. Percent of seed capsules produced by hand pollination treatment. Refer to Table 5 for hand pollination treatments.

	Seed capsules produced (%)			
Treatment	Means	± S.E.	Student Newman-Keuls mean comparison	N
10	0.0	0.0	A	2
19	0.0	0.0	A	2
15	6.3	6.3	A	2
9	18.2	5.4	AB	2
8	33.3	0.2	BC	2
14	43.8	6.3	D C	2
6	44.9	0.6	D C	2
7	53.3	13.3	DEC	2
17	59.7	10.7	DE	2
2	71.9	14.7	EF	2
5	84.5	10.7	F	2
13	86.9	4.4	F	2
3	87.9	2.1	F	2
4	89.5	0.5	F	2
1	91.4	1.4	F	2
12	99.1	0.9	F	2
18	99.5	0.5	F	2
11	100.0	0.0	F	2
16	100.0	0.0	F	2

Table 8. Results of analysis of variance of seed capsule volume by hand pollination treatment. Refer to Table 5 for hand pollination treatments.

Source	DF	S.S.	M.S.	F-Value	P-Value
Grand Mean(38) =26.5					
Total (adj. for mean)	37	5847.2			
Treatment	18	5714.0	317.5	45.3	0.0001
Residual	19	133.2	7.0		

Table 9: Mean seed capsule volume by hand pollination treatment. Refer to Table 5 for hand pollination treatments.

	Seed capsule volume (mm^3)			
Treatment	Means	± S.E.	Student Newman-Keuls Mean Comparison	N
10	0.0	0.0	A	2
19	0.0	0.0	A	2
6	11.2	0.5	B	2
4	11.3	0.8	B	2
3	19.9	0.6	C	2
5	23.5	1.3	CD	2
1	29.3	0.8	DE	2
2	29.6	0.3	DE	2
8	29.8	1.2	DE	2
13	29.9	0.3	DE	2
14	30.9	0.4	DE	2
12	31.9	0.1	DE	2
16	32.0	2.4	DE	2
7	32.4	1.2	DE	2
9	34.9	1.1	E	2
18	35.8	3.9	E	2
17	36.6	4.9	E	2
11	38.1	1.3	E	2
15	46.1	3.5	F	2

Table 10. P- values generated from ANOVA of the effects of *Brachypterolus pulicarius* and plant age (at time of insect release) on Dalmatian toadflax, growth and reproduction.

	Height	Flower stems	Primary branches	Secondary branches	Flower buds	Flowers	Capsules	Seeds	Seed weight	Capsule volume
	(cm)	----------(Number per plant)----------							(mg)	(mm^3)
Greenhouse -1992										
B. pulicarius	0.0001	0.8918	0.0001	0.0001	0.0025	0.0002	0.0001	0.0001	0.0019	0.0277
Plant age	0.0002	0.1371	0.1219	0.0129	0.0004	0.0001	0.0001	0.0001	0.0918	0.1746
B. pulicarius X plant age	0.0009	0.3126	0.3729	0.0488	0.2565	0.1690	0.0500	0.0071	0.6699	0.1192
Greenhouse - 1993										
B. pulicarius	0.0478	0.0835	0.0001	0.0065	0.5461	0.0479	0.0159	0.0023	0.0164	0.0265
Plant age	0.0004	0.0002	0.2323	0.7214	0.0004	0.0001	0.0001	0.0001	0.0237	0.0795
B. pulicarius X plant age	0.1248	0.7343	0.9038	0.8927	0.8554	0.3441	0.0412	0.0100	0.1527	0.0725
Field - 1993										
B. pulicarius	0.0009	0.7127	0.0002	0.0001	0.4747	-----	-----	-----	-----	-----
Plant age	0.0001	0.3496	0.0851	0.0235	0.0012	-----	-----	-----	-----	-----
B. pulicarius X plant age	0.1758	0.7418	0.0910	0.0416	0.8568	-----	-----	-----	-----	-----

Table 11. Main effects of *Brachypterolus pulicarius* and plant age (at the time of release) on Dalmatian toadflax height in 1993.

	Mean height per plant (cm)	
	Greenhouse	Field
B. pulicarius		
Absent	54.3	60.6
Present	46.9	47.3
Standard error (model)	2.7	4.6
Plant age		
3 months	37.5	37.8
6 months	54.0	56.7
12 months	60.3	67.3
Standard error (model)	2.9	5.0

Table 12. Main effects of *Brachypterolus pulicarius* on the number of Dalmatian toadflax primary branches.

	Mean number of primary branches per plant		
	1992 Greenhouse	1993 Greenhouse	1993 Field
B. pulicarius			
Absent	6.3	7.6	6.6
Present	13.3	33.3	24.4
Standard error (model)	2.3	8.1	7.1

Table 13. Main effects of *Brachypterolus pulicarius* and plant age (at the time of release) on the flower buds and flowers of Dalmatian toadflax.

	Mean number of flower buds per plant			Mean number of flowers per plant		
	1992	1993		1992	1993	
	Greenhouse	Greenhouse	Field	Greenhouse	Greenhouse	Field
B. pulicarius						
Absent	185.5	NS	NS	48.6	134.6	------
Present	157.8	NS	NS	25.0	75.6	------
Standard error (model)	11.0	NS	NS	6.1	18.1	------
Plant age						
3 months	42.1	33.7	46.0	18.6	6.3	------
6 months	176.6	275.6	133.4	38.9	68.4	------
12 months	191.6	740.4	222.8	53.0	253.4	------
Standard error (model)	9.6	95.5	36.9	5.2	33.3	------

Table 14. Main effects of *Brachypterolus pulicarius* on the seed weight and seed capsule volume of Dalmatian toadflax.

	Mean seed weight per capsule (mg)		Mean seed capsule volume per plant (mm^3)	
	1992	1993	1992	1993
B. pulicarius				
Absent	0.0164	0.0147	18.5	24.4
Present	0.0092	0.0098	23.9	16.6
Standard error (model)	0.0021	0.0016	2.4	3.1

Table 15. Site location, population code, host plant, and collection date for each population of *Brachypterolus pulicarius* sampled.

Location	Population code	Host plant	Collection date
Kamloops, British Columbia, Canada Research Station	DT-1	Dalmatian toadflax	05-25-1993
Kamloops, British Columbia, Canada Community College	DT-2	Dalmatian toadflax	05-26-1993
Kamloops, British Columbia, Canada Railroad right-of-way	DT-3	Dalmatian toadflax	05-25-1993
Kamloops, British Columbia, Canada Gravel pit	DT-4	Dalmatian toadflax	05-26-1993
Butte, Montana I-90	YT-1	Yellow toadflax	06-12-1993
Ovando, Montana US-200	YT-2	Yellow toadflax	06-03-1993
Radersburg, Montana 14 Mile Rd.	YT-3	Yellow toadflax	06-17-1993
Pipestone, Montana Homestake Pass	YT-4	Yellow toadflax	06-12-1993
Radersburg, Montana South Crow Creek	YT-5	Yellow toadflax	06-17-1993

Table 16. Enzyme stains and buffer systems used in isozyme analysis of *Brachypterolus pulicarius*.

Enzyme System	Abbreviation	Enzyme Commission Number	Number of Loci[a]	Buffer System[b]
Adenylate kinase	AK	2.7.4.3	2	TM 7.4
Aspartate amino-transferase	AAT	2.6.1.1	1	LiOH
α- andβ-Esterase	EST	3.1.1.1	1	TM 7.4
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1.2.1.12	1	LiOH
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	3	TM 7.4
Glucose phosphate isomerase	GPI	5.3.1.8	2	TM 7.4
Hexokinase	HK	2.7.1.1	2	TM 7.4
Isocitrate dehydrogenase (NADP)	IDH	1.1.1.42	1	LiOH
Lactate dehydrogenase	LDH	1.1.1.30	1	LiOH
Malate dehydrogenase	MDH	1.1.1.37	3	TC 8.0
Malic enzyme	ME	1.1.1.40	3	TC8.0
Mannose-6-phosphate isomerase	MPI	5.3.1.8	1	TC 8.0
Phosphoenolpyruvate carboxylase	PEP	4.1.1.31	1	TC 8.0
Phosphoglucomutase	PGM	2.7.5.1	1	TM 7.4
Sorbitol dehydrogenase	SDH	1.1.1.14	1	LiOH
Superoxide dismutase	SOD	1.15.1.1	1	TC 8.0
Xanthine dehydrogenase	XDH	1.1.1.204	1	TC 8.0

[a] Number of bands per phenotype.
[b] Electrophoresis buffer.

Table 17. Gel/electrode electrophoresis buffer systems used in this study.

Buffer	Step	Reagent	Quantity	Source
LiOH[a]	Electrode buffer	Lithium hydroxide	2.52 g	I-4256[d]
		Boric acid	18.55 g	B-0252
		Water, make up to	1000.0 ml	
	Gel buffer	Trisma base	3.63 g	T-1503
		Citric acid	0.96 g	C-7129
		Electrode buffer	10.0 ml	
		Water, make up to	1000.0 ml	
	Gel (quantity 1)	Starch	60 g	S-4501
		LiOH gel buffer	500 ml	
TC 8.0[b]	Electrode buffer	Trisma base	75.64 g	T-1503
		Citric acid	30.0 g	C-7129
		Water, make up to	1000.0 ml (adjust pH to 8.0)	
	Gel buffer	Electrode buffer	16.6 ml	
		Water, make up to	500 ml (adjust pH to 8.0)	
	Gel (quantity 1)	Starch	60 g	S-4501
		TC 8.0 gel buffer	500 ml	
TM 7.4[c]	Solution I	Tris	24.2 g (0.1 M)	T-1503
		Malic anhydride	16.2 g (0.08 M)	M-1000
		Water, make up to	1000.0 ml	
	Solution II	NaOH	8.0 g (0.1 M)	S-5881
		Water, make up to	1000.0 ml	
	Electrode buffer	Solution I	1600.0 ml	
		Solution II	add until pH 7.4	
	Gel buffer	Solution I	125.0 ml	
		Solution II	add until pH 7.4	
		Water, make up to	500.0 ml	
	Gel (quantity 1)	Starch	60 g	S-4501
		Gel buffer	500 ml	

[a] Vawter and Brussard 1975.
[b,c] Pasteur et al. 1988.
[d] Source numbers correspond to 1993 Sigma Chemical Company catalogue.

Table 18. Staining protocols for enzymes used in this study.

Enzyme	Reagent	Quantity	Source
Ak[a]	- Glucose	200.0 mg	G-8270[b]
	- Adenosine diphosphate	100.0 mg	A-0127
	- LiOH gel buffer (see Table 17)	80.0 ml	
	- G6PDH	60.0 units	G-7750
	- β-Nicotinamide adenine dinucleotide (NADP)	10.0 ml	N-0505
	- 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)	10.0 ml	M-2128
	- Phenazine methosulfate (N-Methyldibenzopyrazine methyl sulfate salt)(PMS)	3.0 ml	P-9625
AAT	- AAT Buffer (see Table 19)	50.0 ml	
	- Fast garnet GBC salt	1000.0 mg	F-0875
	- Fast blue salt (optional)	200.0 mg	F-025
EST	- α-Naphthyl acetate	50.0 mg	N-6875
	- β-Naphthyl acetate	50.0 mg	N-8505
	(dissolve acetates in 1.0 ml acetone)		
	- Fast blue BB salt	50.0 mg	F-0250
	- LiOH gel buffer	80.0 ml	
GAPDH	- Frutose-1,6-diphosphate	55.0 mg	752-1
	- Aldolase	100.0 units	A-6253
	- LiOH gel buffer	80.0 ml	
	- β-Nicotinamide adenine dinucleotide (NAD)	20.0 mg	N-7004
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625
G6PDH	- Glucose-6-phosphate	400.0 mg	G-7879
	- LiOH gel buffer	80.0 ml	
	- NADP	10.0 mg	N-0505
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625
GPI	- Frutose-6-phosphate	50.0 mg	F-3627
	- LiOH gel buffer	80.0 ml	
	- G6PDH	60.0 units	G-7750
	- NADP	10.0 mg	N-0505
	- MTT	20.0 mg	M-2128
	- PMS	3.0 mg	P-9625

Table 18. (cont.).

Enzyme	Reagent	Quantity	Source
HK	- Tris-B (see Table 19)	10.0 ml	
	- Water	90.0 ml	
	- $MgCl_2$ crystals	1000.0 mg	M-0250
	- Glucose	200.0 mg	G-8720
	- Adenosine 5'-triphosphate (ATP)	125.0 mg	A-5394
	- G6PDH	60.0 units	G-7750
	- NADP	10.0 mg	N-0505
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625
IDH	- Isocitric acid	75.0 mg	I-1252
	- $MgCl_2$ crystals	1.0 g	M-0250
	- LiOH gel buffer	80.0 ml	
	- NADP	10.0 mg	N-0505
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625
LDH	- Lactate (0.5 M)	10.0 ml	
	- LiOH gel buffer	80.0 ml	
	- NAD	20.0 mg	N-7004
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625
MDH	- Malic acid (pH 7.0) (see Table 19)	10.0 ml	
	- LiOH gel buffer	80.0 ml	
	- NAD	20.0 mg	N-7004
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625
ME	- Malic acid (pH 7.0)	10.0 ml	
	- LiOH gel buffer	80.0 ml	
	- NADP	10.0 mg	N-0505
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625
MPI	- Mannose-6-phosphate	35.0 mg	M-8754
	- LiOH gel buffer	80.0 ml	
	- G6PDH	60.0 units	G-7750
	- Phosphoglucose isomerase (PGI)	100.0 units	P-9010
	- NADP	10.0 mg	N-0505
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625

Table 18. (cont.).

Enzyme	Reagent	Quantity	Source
PEP	- Glycyl-leucine	50.0 mg	G-2002
	- Peroxidase	20.0 mg	P-8125
	- Amino acid oxidase	5.0 mg	V-7000
	- O-dianisidine	25.0 mg	D-3252
	- LiOH gel buffer	80.0 ml	
PGM	- Glucose-1-phosphate	100.0 mg	G-7000
	- LiOH gel buffer	80.0 ml	
	- G6PDH	60.0 units	G-7750
	- NADP	10.0 mg	N-0505
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625
SDH	- Sorbitol	10.0 ml	S-1876
	- LiOH gel buffer	80.0 ml	
	- NAD	20.0 mg	N-7004
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625
SOD	- LiOH gel buffer	80.0 ml	N-7004
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625
XDH	- Hypoxanthine	20.0 mg	H-9377
	- LiOH gel buffer	80.0 ml	
	- NAD	20.0 mg	N-7004
	- MTT	10.0 mg	M-2128
	- PMS	3.0 mg	P-9625

[a] Recommended name of enzyme and corresponding E.C. number listed in Table 16.
[b] Source numbers correspond to 1993 Sigma Chemical Company catalogue.

Table 19. Buffers used in enzyme staining protocols.

Buffer	Reagent	Quantity	Source
AAT	- α-Ketoglutarate	0.75 g	K-1750[a]
	- L-aspartate	2.75 g	A-9006
	- EDTA	1.00 g	ED2SS
	- PVP-40	10.0 g	PVP-40
	- NaH_2PO_4	15.0 g	S-0751
	- Na_2HPO_4	15.0 g	S-0876
	- Water, make up to	1000.0 ml	
Malic Acid (ph 7.0)	- D-L-Malic acid	33.5 g	M-1000
	- $NaHCO_3$	55.0 g	S-8875
	- Water, make up to	50.0 ml	
Tris-B (pH 8.5)	- Tris-HCl	2.21 g	T-3253
	- Water, make up to	50 ml	

[a] Source numbers correspond to 1993 Sigma Chemical Company catalogue.

Table 20. Allelic frequencies for 27 loci from *B. pulicarius* collected off of Dalmatian and yellow toadflax.

Locus	Allele	DT-1[a]	DT-2	DT-3	DT-4	YT-1	YT-2	YT-3	YT-4	YT-5
AK-1	1	—	—	—	—	0.06	—	—	—	—
	2	1.00	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00
	n	20	18	21	23	17	20	23	17	20
AK-2	1	—	0.03	0.048	—	0.06	—	—	—	—
	2	1.00	0.97	0.95	1.00	0.94	1.00	1.00	1.00	1.00
	n	20	18	21	23	17	20	23	17	20
AAT	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	20	18	8	23	8	6	23	8	20
EST	1	—	—	—	1.00	0.13	0.13	0.25	1.00	—
	2	1.00	1.00	1.00	—	0.88	0.87	0.75	—	1.00
	n	20	9	15	8	7	10	6	6	20
GAPDH	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	9	16	10	10	16	6	10	12	12
G6PDH-1	1	0.2	—	—	0.13	—	—	—	—	—
	2	0.8	1.00	1.00	0.87	1.00	1.00	1.00	1.00	1.00
	n	10	9	12	10	8	8	6	12	10
G6PDH-2	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	20	18	12	23	8	20	20	17	23
G6PDH-3	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	20	18	12	23	8	20	20	17	23
GPI-1	1	0.1	—	—	—	0.06	—	—	—	—
	2	0.95	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00
	n	20	18	12	23	8	20	23	17	20
GPI-2	1	—	—	—	—	0.13	—	—	—	—
	2	1.00	1.00	1.00	1.00	1.01	1.00	1.00	1.00	1.00
	n	10	9	12	16	8	17	16	12	10
HK-1	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	28	18	21	23	17	20	23	17	20
HK-2	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	28	18	21	23	17	20	23	17	20
IDH	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	10	7	6	6	6	8	7	6	10

Table 20. (Cont.)

Locus	Allele	DT-1	DT-2	DT-3	DT-4	YT-1	YT-2	YT-3	YT-4	YT-5
LDH	1	—	—	—	—	—	—	0.04	0.16	—
	2	1.00	1.00	1.00	1.00	1.00	1.00	0.95	0.83	1.00
	n	10	4	7	23	6	11	23	7	10
MDH-1	1	—	—	0.05	—	0.06	—	—	0.08	—
	2	1.00	1.00	0.95	1.00	0.94	1.00	1.00	0.92	1.00
	n	20	18	21	23	17	20	23	17	20
MDH-2	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	20	18	21	23	17	20	23	17	20
MDH-3	1	—	—	—	—	—	0.06	—	—	—
	2	1.00	1.00	1.00	1.00	1.00	0.94	1.00	1.00	1.00
	n	20	18	21	23	17	20	23	17	20
ME-1	1	—	—	—	—	0.06	—	—	—	—
	2	1.00	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00
	n	20	18	21	23	17	20	23	17	20
ME-2	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	20	18	21	23	17	20	23	17	20
MPI	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	20	9	13	7	6	17	7	17	20
PEP	1	0.8	0.78	0.52	0.59	0.2	0.25	0.35	0.33	0.65
	2	0.125	0.22	0.27	0.26	0.8	0.75	0.65	0.54	0.25
	3	0.075	—	0.27	0.15	—	—	—	—	0.1
	n	20	9	13	23	5	8	23	17	20
PGM-1	1	—	—	—	—	—	—	—	0.143	—
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.857	1.00
	n	10	9	7	6	6	17	8	12	10
PGM-2	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	20	18	21	23	9	12	23	17	20
PGM-3	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	20	18	21	23	9	12	23	17	20
SDH	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	10	18	21	23	17	20	23	17	10
SOD	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	20	18	13	7	4	7	7	17	20

Table 20. (Cont.)

Locus	Allele	DT-1	DT-2	DT-3	DT-4	YT-1	YT-2	YT-3	YT-4	YT-5
XDH	1	—	—	—	—	—	0.06	0.13	—	0.11
	2	1.00	1.00	0.81	0.45	0.71	0.88	0.87	0.92	0.89
	3	—	—	0.19	0.55	0.29	0.06	—	0.08	—
	n	18	15	21	20	17	17	15	12	9

[a] Refer to Table 15 to determine the site location, population code, host plant, and collection date for each population of *B. pulicarius* sampled.
n, number of specimens analyzed for a given *B. pulicarius* population.

Table 21. Matrix of Nei's genetic identity (above diagonal lines) and Nei's genetic distance (below diagonal lines: Nei 1972) for populations of *Brachypterolus pulicarius*.

	Population								
Plant spp./site codes	YT-1	YT-3	YT-5	DT-3	DT-1	DT-2	DT-4	YT-2	YT-4
YT-1[1]	———	0.9949	0.9862	0.9895	0.9785	0.9828	0.9861	0.9970	0.9931
YT-3	0.0051	———	0.9928	0.9917	0.9865	0.9902	0.9833	0.9987	0.9954
YT-5	0.0139	0.0072	———	0.9980	0.9972	0.9990	0.9899	0.9914	0.9941
DT-3	0.0106	0.0083	0.0020	———	0.9942	0.9960	0.9940	0.9916	0.9943
DT-1	0.0217	0.0136	0.0028	0.0058	———	0.9981	0.9981	0.9844	0.9889
DT-2	0.0173	0.0098	0.0010	0.0040	0.0019	———	0.9981	0.9886	0.9923
DT-4	0.0140	0.0168	0.0101	0.0060	0.0129	0.0129	———	0.9836	0.9859
YT-2	0.0030	0.0013	0.0086	0.0084	0.0157	0.0115	0.0165	———	0.9962
YT-4	0.0069	0.0046	0.0059	0.0057	0.0112	0.0077	0.0142	0.0038	———

[1]YT, yellow toadflax; DT, Dalmatian toadflax. The number following the host plant abbreviation corresponds to the respective collection site (see Table 15 for additional *B. pulicarius* population code information).

APPENDIX B

FIGURES 1-9

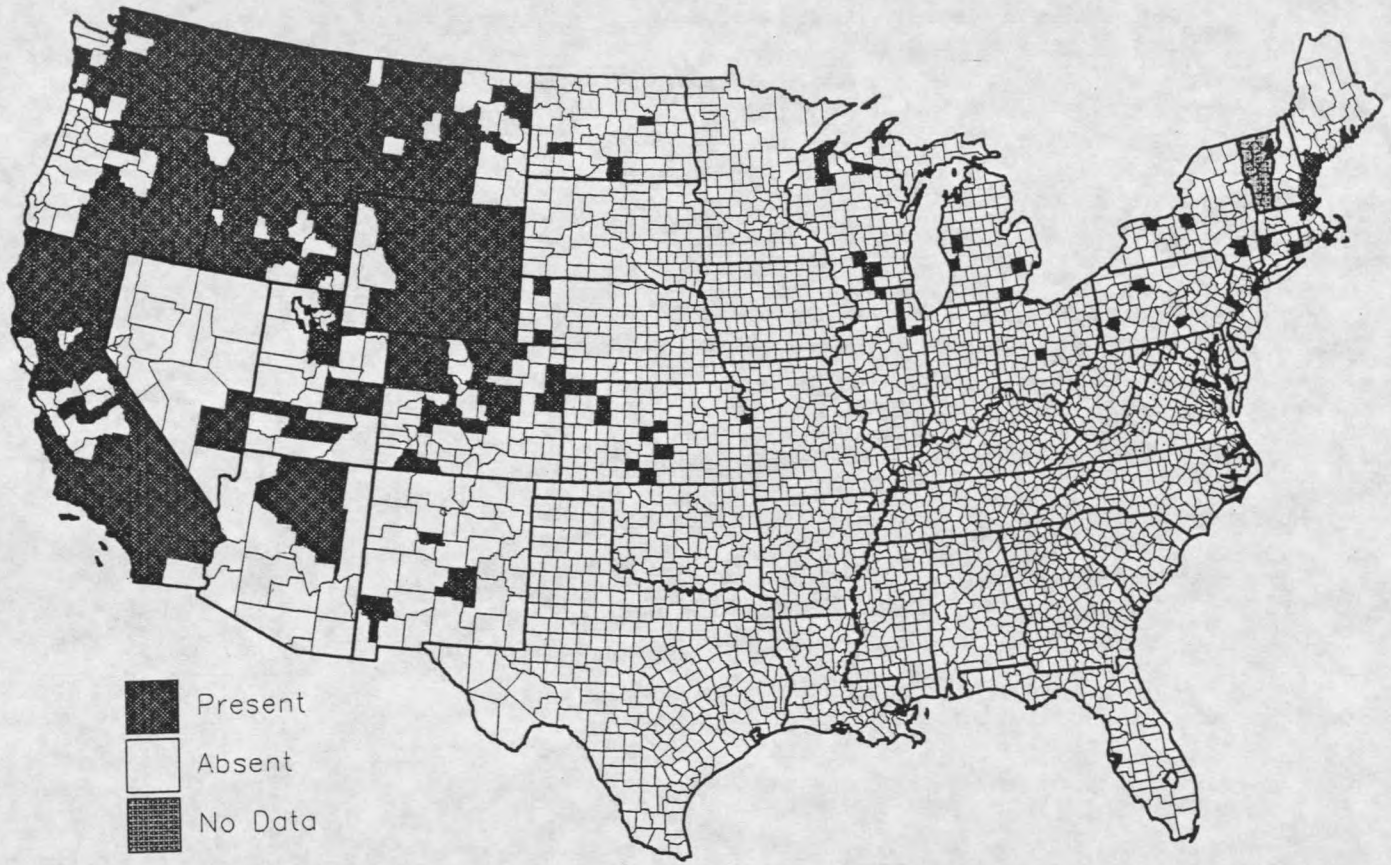
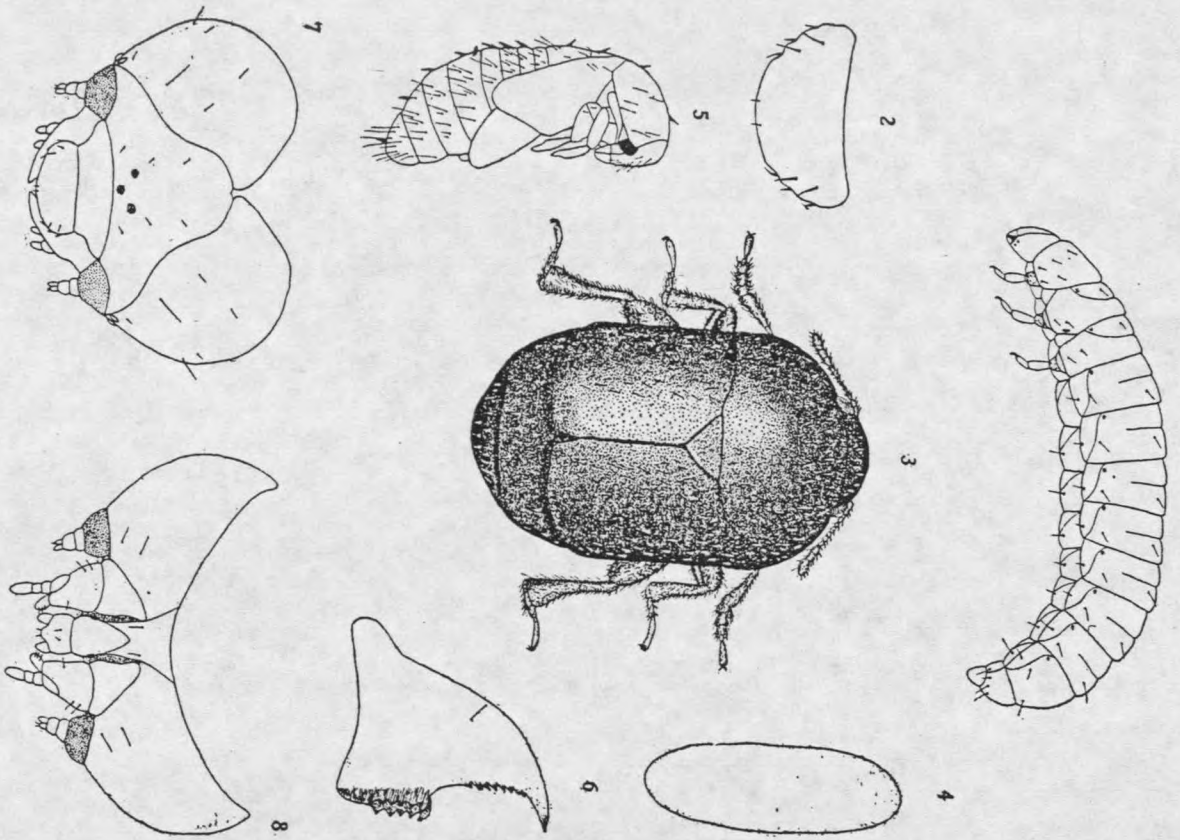


Figure 1. 1992 Distribution of Dalmatian toadflax in the United States. Map of the counties in the United States reporting the presence or absence of Dalmatian toadflax. Compiled by Diana Cooksey, Montana State University, Dept. of Plant, Soil, and Environmental Sciences. Data courtesy of Cooperative Agricultural Pest Survey (CAPS), USDA-APHIS-PPQ



Key: 1) Larva, 2)Labrum of larva, 3) Adult, 4) Egg, 5) Pupa, 6) Mandible of larva, 7) Dorsal aspect of head of larva, 8) Ventral aspect of head of larva.

Figure 2. *Brachypterolus pulicarius* (Hervey 1927).

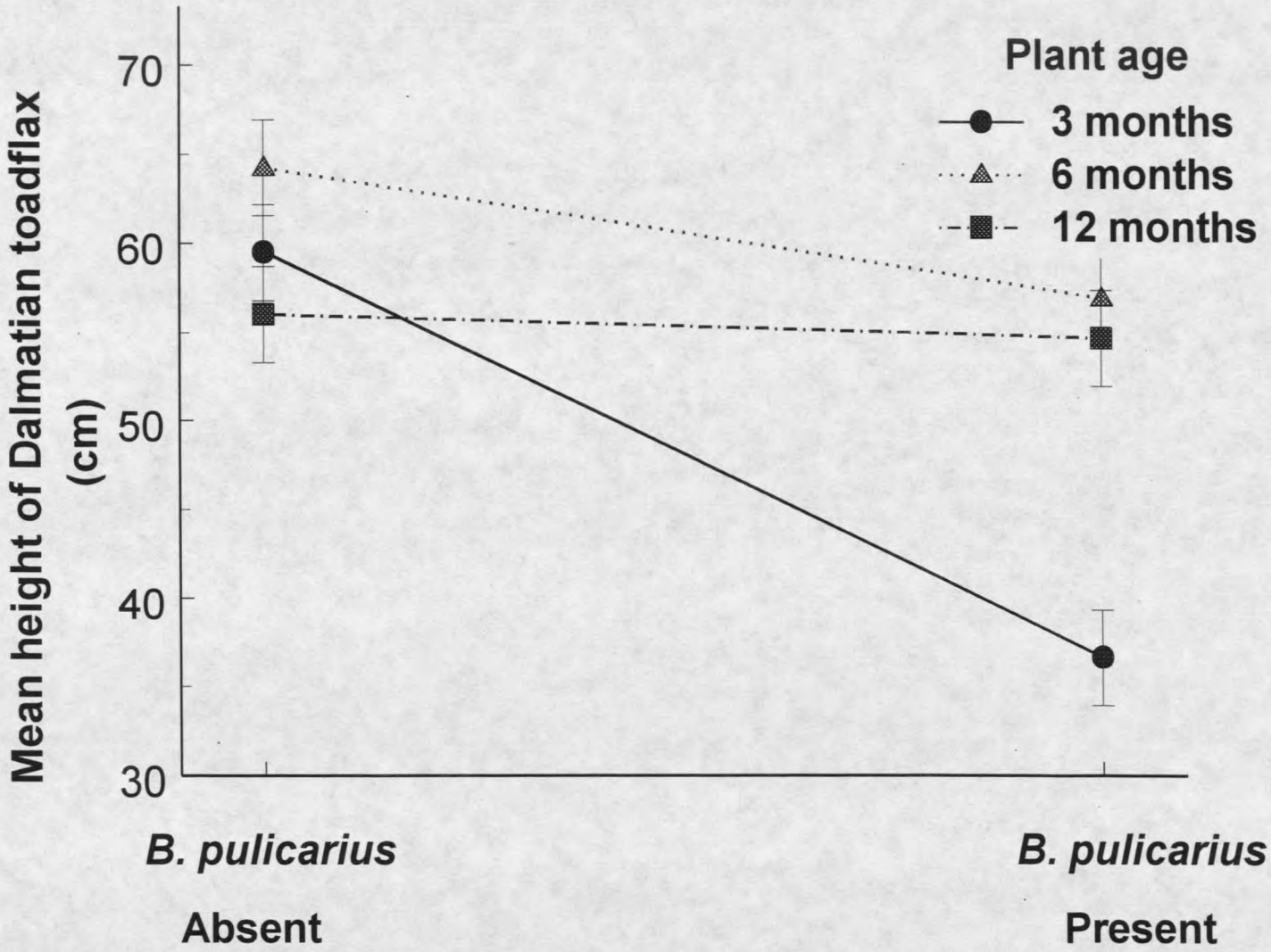


Figure 3. Effects of *B. pulicarius* on the mean height of Dalmatian toadflax plants (three, six, and 12 months old at the time of insect release) in 1992.

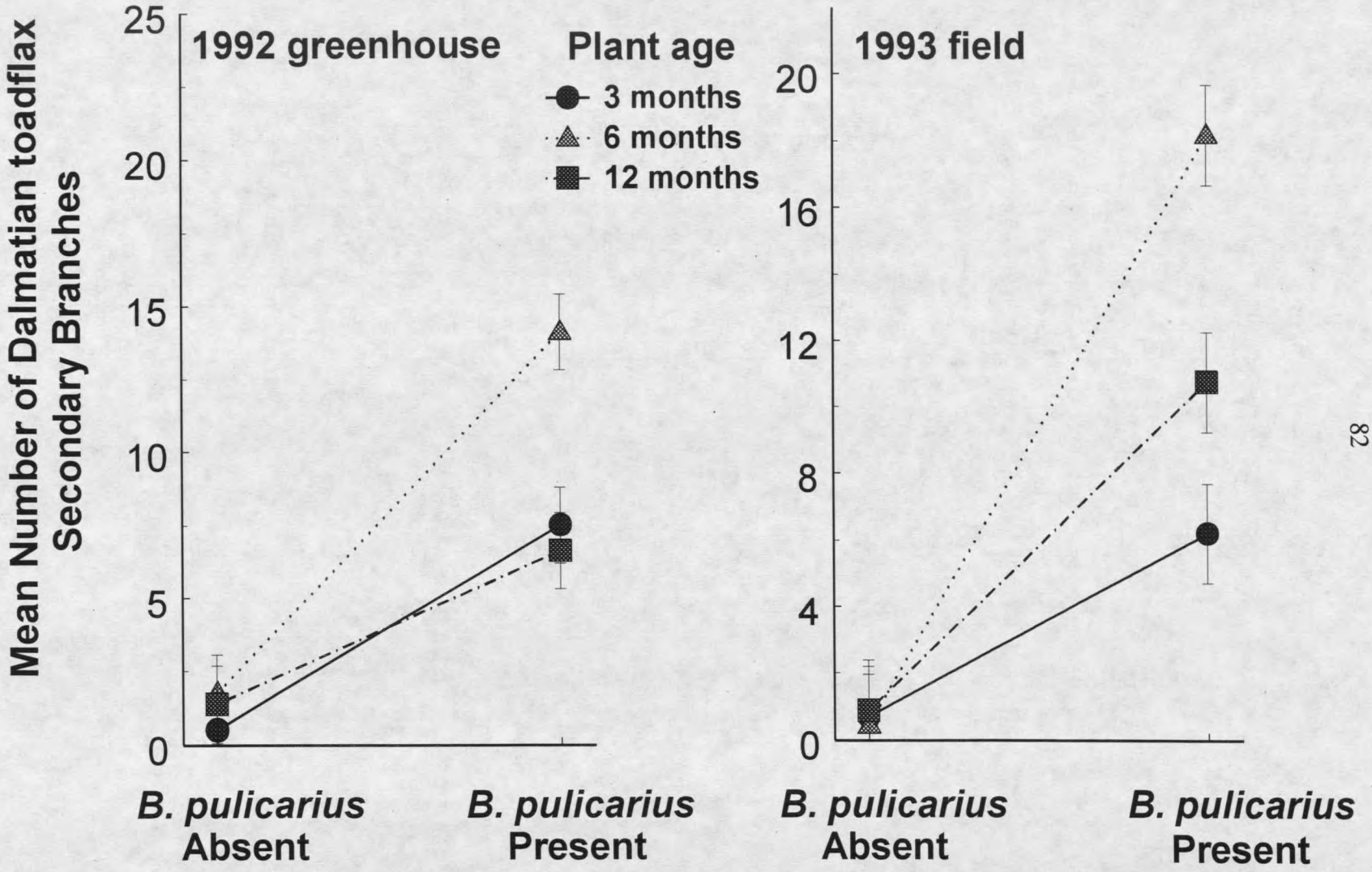


Figure 4. Effects of *B. pulicarius* on the mean number of secondary branches of Dalmatian toadflax plants (three, six, and 12 months old at the time of insect release) in the 1992 greenhouse and 1993 field studies.

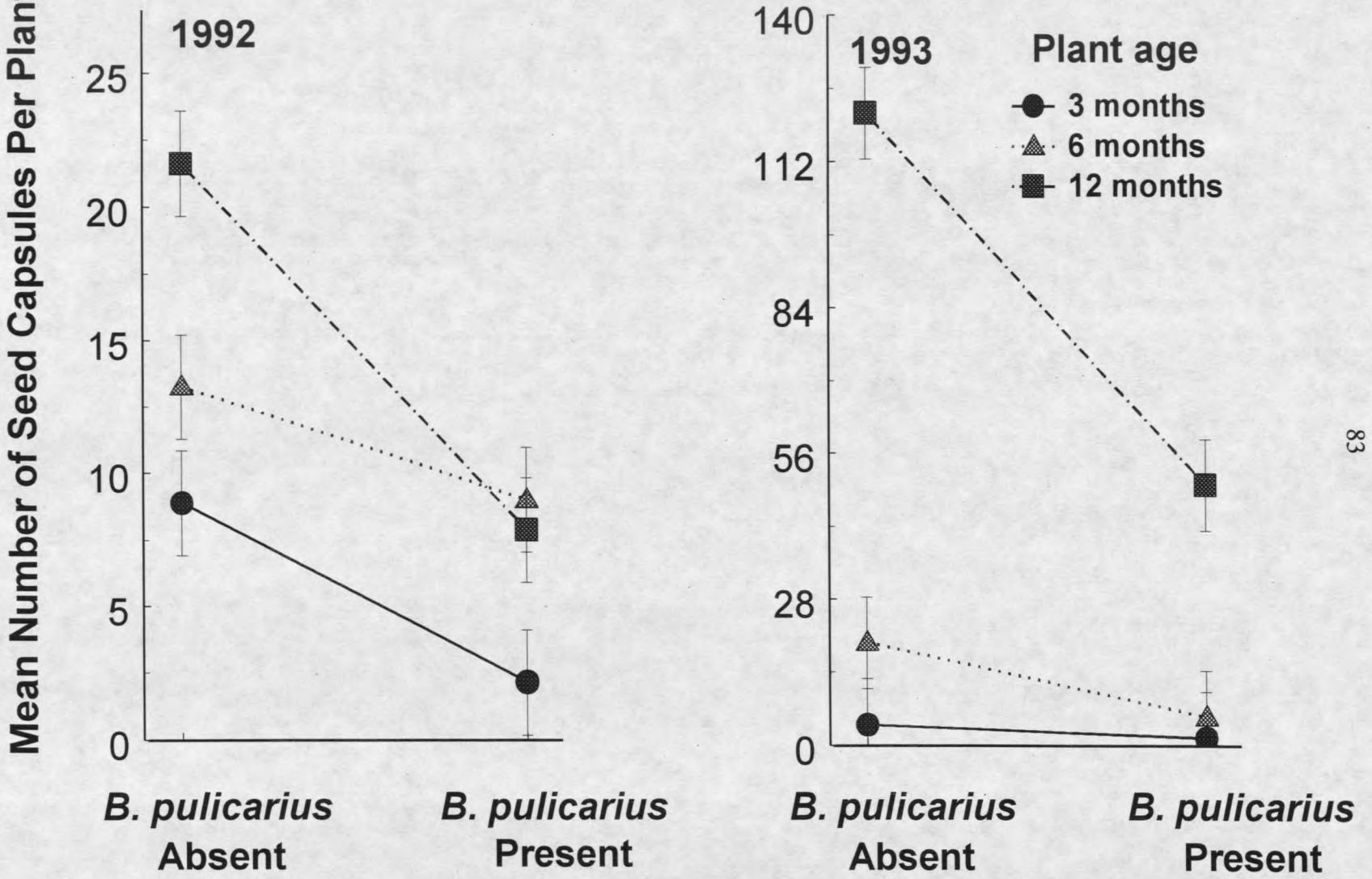


Figure 5. Effects of *B.pulicarius* on the mean number of seed capsules produced on Dalmatian toadflax plants (three, six, and 12 months old at the time of insect release) in the 1992 and 1993 greenhouse studies.

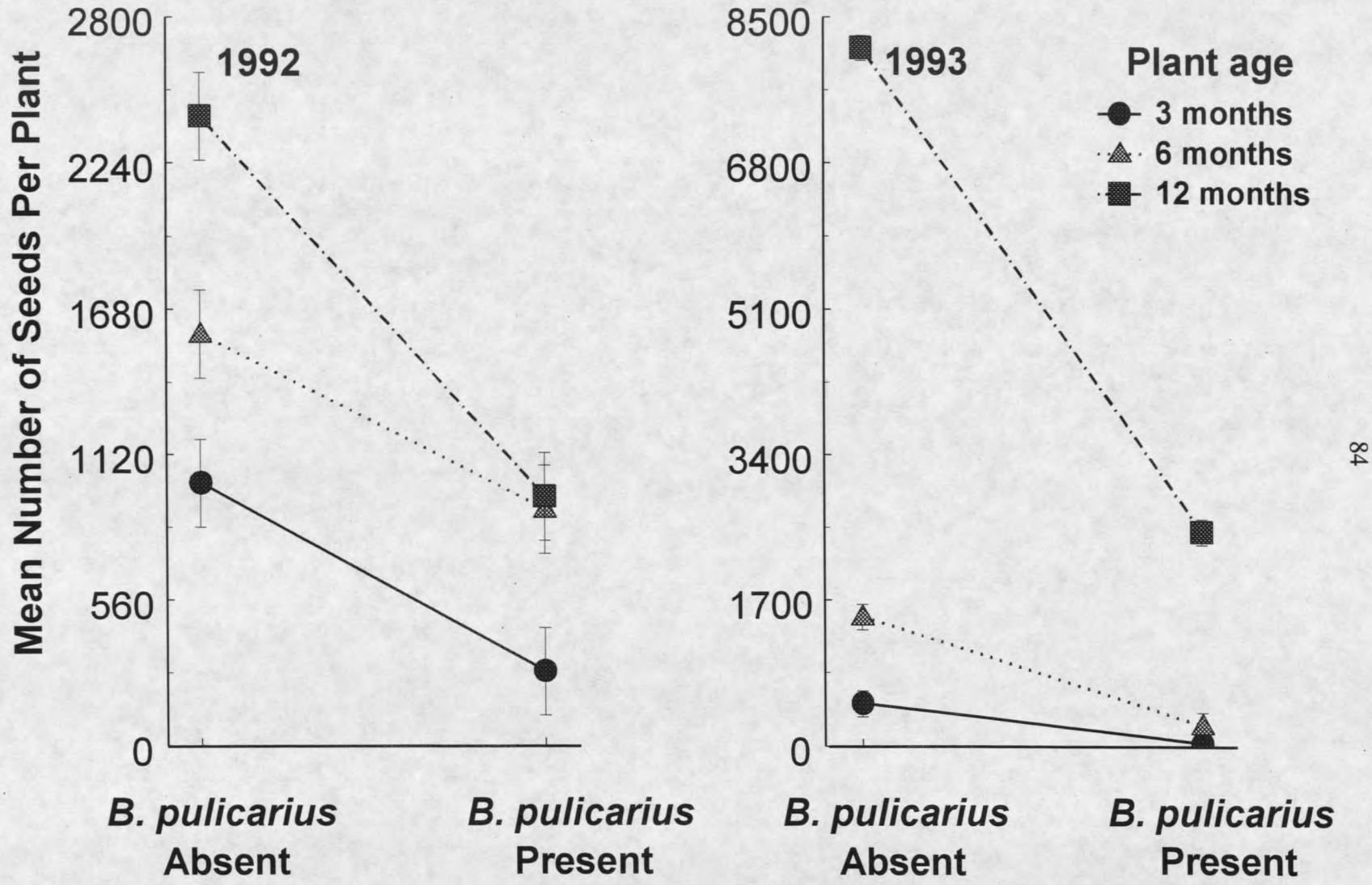


Figure 6. Effects of *B.pulicarius* on the mean number of seeds produced on Dalmatian toadflax plants (three, six, and 12 months old at the time of insect release) in the 1992 and 1993 greenhouse studies.

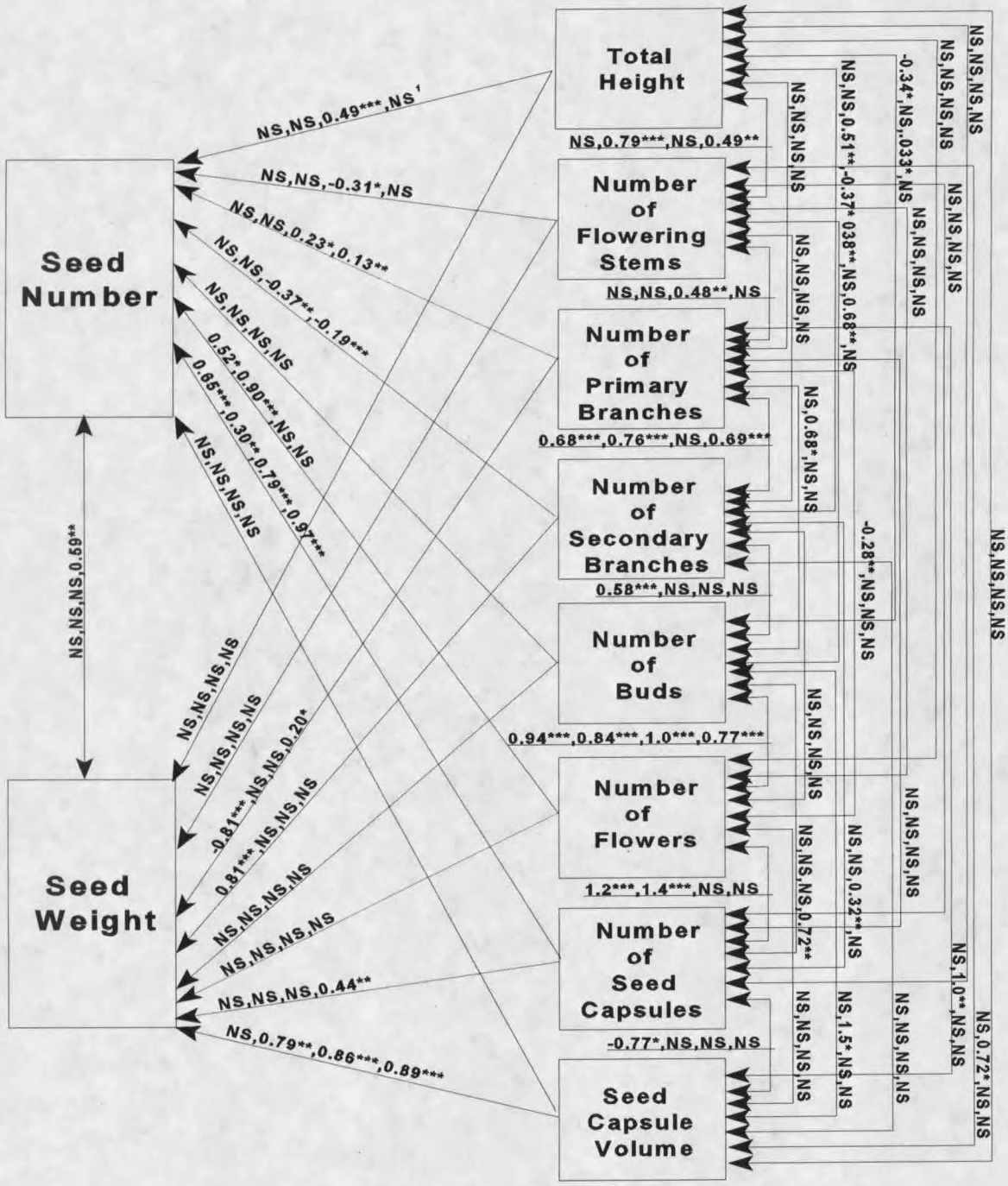


Figure 7. Path diagram of the growth and seed production components of Dalmatian toadflax in the absence and presence of *B. pulicarius*. Path coefficients arranged: *B. pulicarius* absent 1992, *B. pulicarius* absent 1993, *B. pulicarius* present 1992, *B. pulicarius* present 1993.
* $P<0.1$, ** $P<0.05$, *** $P<0.01$

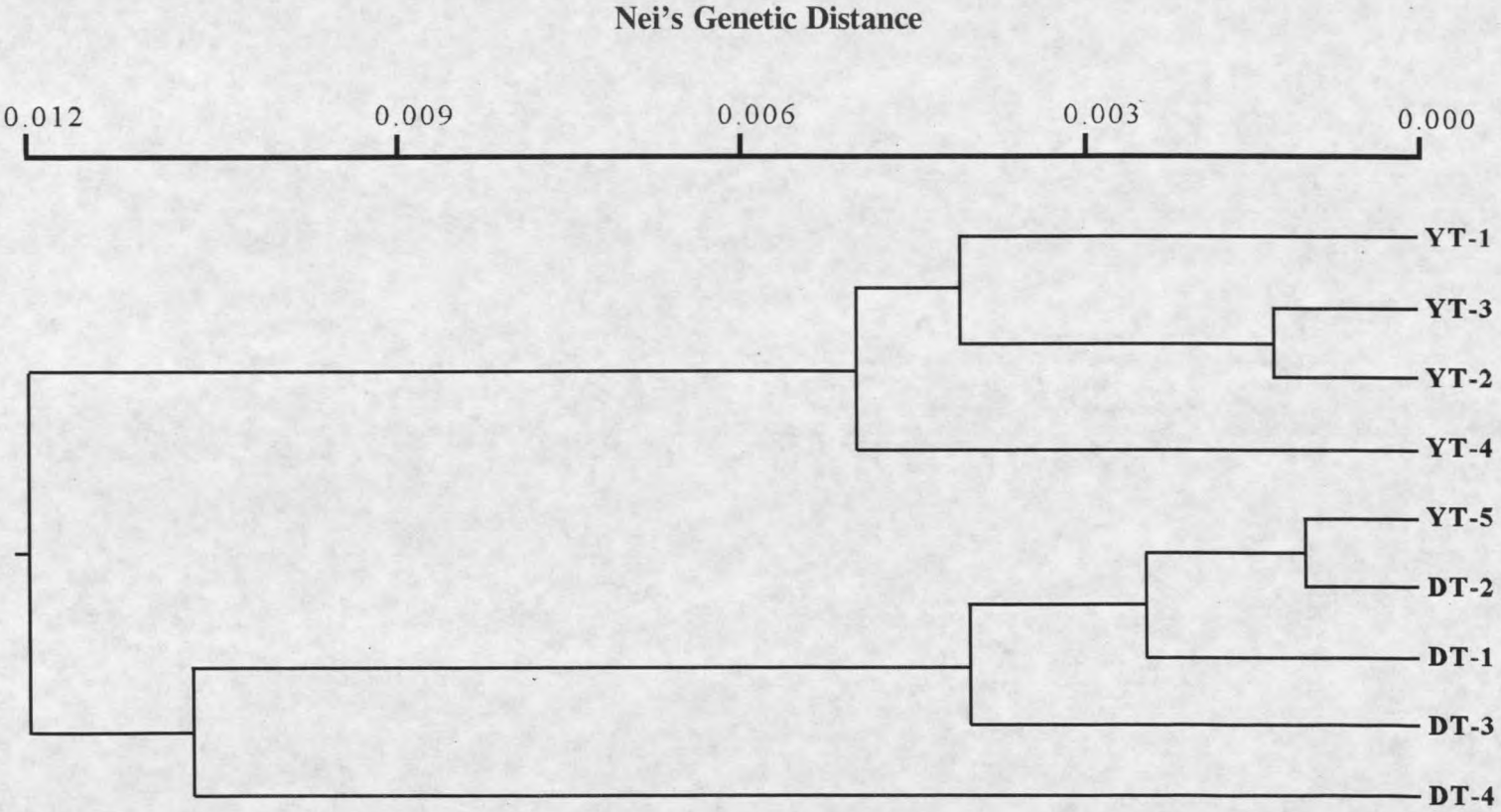


Figure 8. Genetic distance dendrogram, based on Nei's (1972) genetic distances using unweighted pair-group method. Refer to Table 15 for explanation of *B. pulicarius* population codes shown at the end of branches. Software package NTSYS-PC (Rohlf 1993) was used to conduct the data analyses.

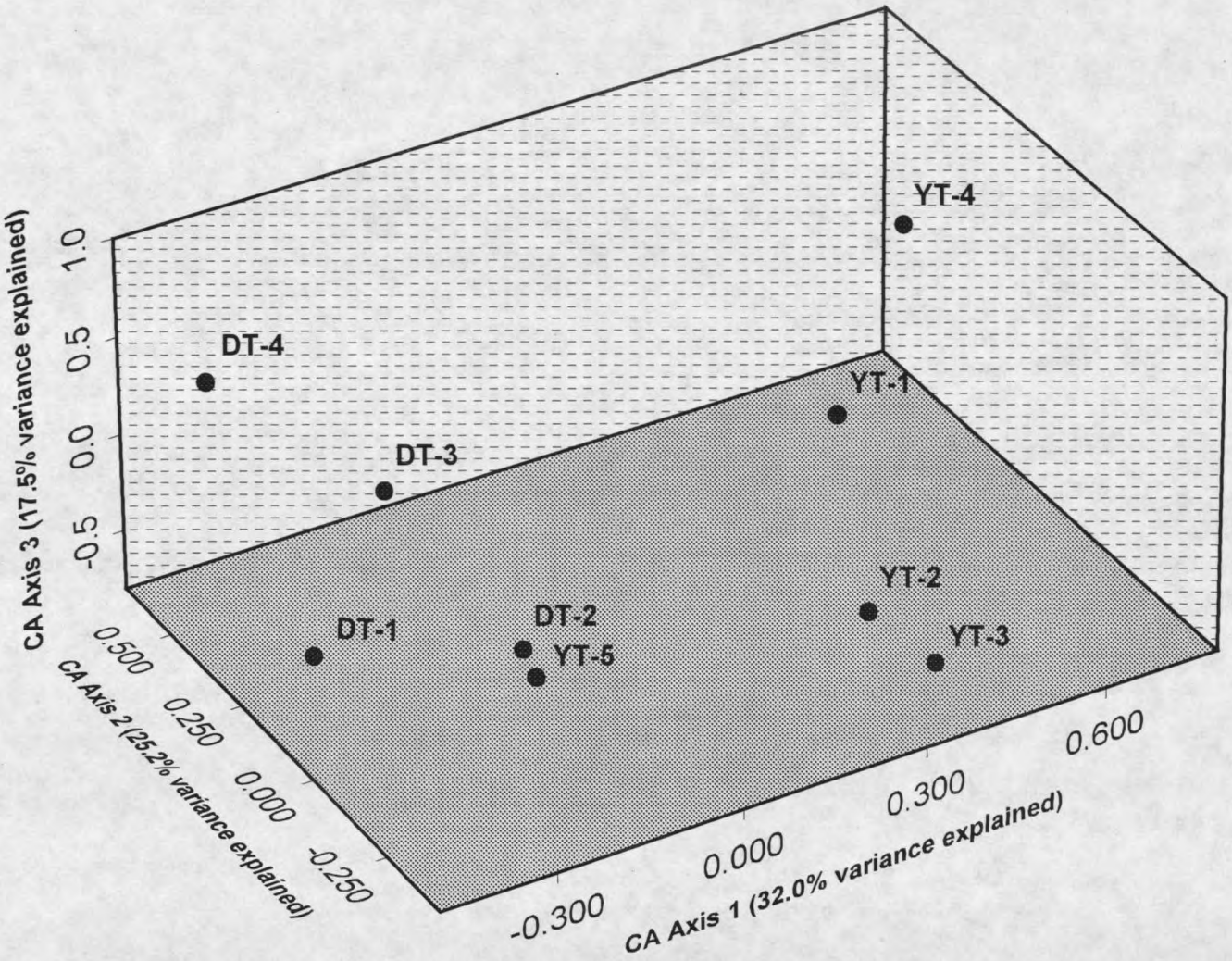


Figure 9. Three-dimensional plot of the correspondence analysis ordination of *Brachypterolus pulicarius* populations based on the frequencies of allelic bands (Statistical package used: CANOCO; Ter Braak 1988). Refer to Table 15 for explanation of *B. pulicarius* population codes.