Fasciation in Invading Common Mullein, *Verbascum thapsus* (Scrophulariaceae): Testing the Roles of Genetic and Environmental Factors[1]

Shahin Ansari[2,3] *and Curtis C. Daehler*[2,4]

Abstract: In Hawai'i, *Verbascum thapsus* L. exhibits high rates of fasciation, which could have ecological and evolutionary consequences for spread of this noxious weed. Fasciated plants produce more seed capsules on average; however, the cause of fasciation in *V. thapsus* is not known. This study investigated whether fasciation in *V. thapsus* has a simple genetic basis, or whether it is caused by physical damage or pathogenic bacteria. Plants derived from self-pollinated fasciated and normal plants were grown in a field common garden and subjected to mechanical damage (simulated herbivory) and natural herbivory. Bacteria cultured from normal and fasciated plants were compared, and field plants were inoculated with a slurry of fasciated tissue. In the common garden, 31% of plants developed fasciation, but fasciation did not follow a simple monogenic pattern of inheritance. Artificial damage substantially reduced fasciation rates; damaged plants were between 1.3 and 32 times less likely to become fasciated, compared with undamaged plants. Bacterial isolates were similar between normal and fasciated plants and no inoculated plants developed fasciation, suggesting that bacteria do not cause fasciation. Fasciated and normal plants often grow less than 1 m apart, indicating that climatic factors are not inducers of fasciation. Localized combinations of environmental conditions in Hawai'i may promote frequent and persistent fasciation.

Analogous to cancer in animals, fasciation is a term used to describe abnormal or uncontrolled proliferation of cells and tissue growth in plants. Fasciation can occur in roots, leaves, flowers, fruits, cotyledons, and underground stems, and it is particularly noticeable in the stems and branches of plants having indeterminate growth (Jones 1935, White 1943). Morphologically, fasciation may be manifested as a flat, banded, or ribbonlike expansion of the stem and/or the inflorescence of a plant. According to Gorter (1965), "true fasciations" result from a lateral expansion of the apical meristem. Linear fasciations are the most common type, wherein the stem, in addition to being broadened in one plane, is often twisted and curled (Lamotte et al. 1988). Besides disorganized tissue growth, a most striking feature of fasciated plants is an unusual increase in weight and/or volume of tissue (White 1943). Fasciation was extensively studied in the early twentieth century under the subject of plant teratology (Wordsell 1915, 1916). Fasciation is widespread geographically and occurs in at least 107 plant families; it has been reported from herbs, shrubs, vines, and trees, although the most striking fasciations probably occur among biennials (White 1943, 1948).

Fasciation in plants may have a genetic basis, or it could be caused by an environmen-

[2] Department of Botany, 3190 Maile Way, Honolulu, Hawai'i 96822.

[3] Current address: SWCA Environmental Consultants, 201 Merchant Street, Honolulu, Hawai'i 96813.

[4] Corresponding author (e-mail: daehler@hawaii.edu).

tal factor or bacterial infection. *Rhodococcus fascians* is a pathogenic bacterium that causes fasciation in several monocots and dicots ranging across 40 families and 86 genera (Putnam and Miller 2007). *Rhodococcus fascians* induces a range of malformations in the host, including leaf deformation, leaf inhibition, witch's brooms, fasciations, and leafy galls (Vereecke et al. 2000).

When fasciation is not caused by bacteria, it often has a genetic basis. Several true-breeding homozygous races of fasciated plants were reported by White (1943, 1948), including in garden peas (*Pisum sativum umbellatum*), cockscomb (*Celosia cristata*), tobacco (*Nicotiana tabacum*), common balsam (*Impatiens balsamina*), annual phlox (*Phlox drummondii*), and Japanese morning glory (*Pharbitis nil*). Several studies have shown that fasciation has a monogenic pattern of Mendelian inheritance (Dwivedi and Singh 1990, Tyagi and Gupta 1991, Knights 1993, Goldman 1998), and a few have identified the specific gene responsible (Wyongyai et al. 1984, Laufs et al. 1998, Tang and Knap 1998). In some natural populations of *Bauhinia* sp. and *Phorodendron* sp., fasciation has become fixed (White 1943), resulting in morphologically distinct populations, perhaps with ecological and evolutionary repercussions.

Compared with the genetic and pathological basis of fasciation, environmental causes of fasciation are less well understood. Damage, herbivory, and increases in available nutrients are a few of the environmental factors that have been suggested to induce fasciation (White 1948). In *Oenothera* species, microscopic injuries caused by a moth (*Mompha* sp.) were regarded as the primary cause of fasciation, and an increase in nutrient availability also increased the number of fasciated plants (Knox 1908). In *Raphanus raphanistrum*, every fasciated shoot was associated with beetle larvae burrowing through the center and then upward to the growing point (Molliard 1900). Nematodes were reported to induce stem fasciation in Henry's lily (*Lilium henryi*) (Stumm-Tegethoff and Linskens 1985). Intentionally injuring the growing tip of wereke (*Ibervillea sonorae*), crushing the young stem of pansies (*Viola tricolor*), and cutting the root tip of fava bean (*Vicia faba*) have all been reported to induce fasciation (White 1948).

Verbascum thapsus L., commonly referred to as common mullein or great mullein, is a Eurasian biennial that is classified as a noxious weed in Hawai'i. It is currently confined to the island of Hawai'i; noxious weed regulations may have helped prevent its establishment on other Hawaiian islands. High rates of extreme fasciation in *V. thapsus* (up to 35%) were first reported in Hawai'i by Juvik and Juvik (1992). Those authors observed fasciation in surveys made during the early 1980s, and fasciation remained common during the course of this study (Figure 1), which documents its persistence in Hawai'i for at least 30 yr.

Fasciation in *V. thapsus* has not been reported in its natural range in Europe, nor is it frequent in its introduced range in North America (Reinartz 1984, Juvik and Juvik 1992, Parker et al. 2003). No fasciation has been documented in introduced populations on the island of La Réunion, which is climatically similar to Hawai'i (Juvik and Juvik 1992).

The cause of fasciation in *V. thapsus* in Hawai'i is not known. This study tested the following three hypotheses: (1) fasciation has a simple genetic basis; (2) mechanical injury predisposes the plants to fasciation; and (3) fasciation is induced by pathogenic bacteria. We also compared capsule production between fasciated and normal *V. thapsus* to test for a potential fitness difference in fasciated plants.

MATERIALS AND METHODS

Seed Collection

Verbascum thapsus has a mixed mating system; the flowers are pollinated by insects, but delayed selfing occurs toward the end of the day in unvisited flowers (Donnelly et al. 1998). Controlled self-pollinations were performed to obtain pure lines derived from normal and fasciated plants and to facilitate genetic analysis. Thirty five each of normal and fasciated, mature *V. thapsus* plants were identified from seven sites along an elevational gradient from 1,829 m to 2,765 m on Mauna Kea (island of
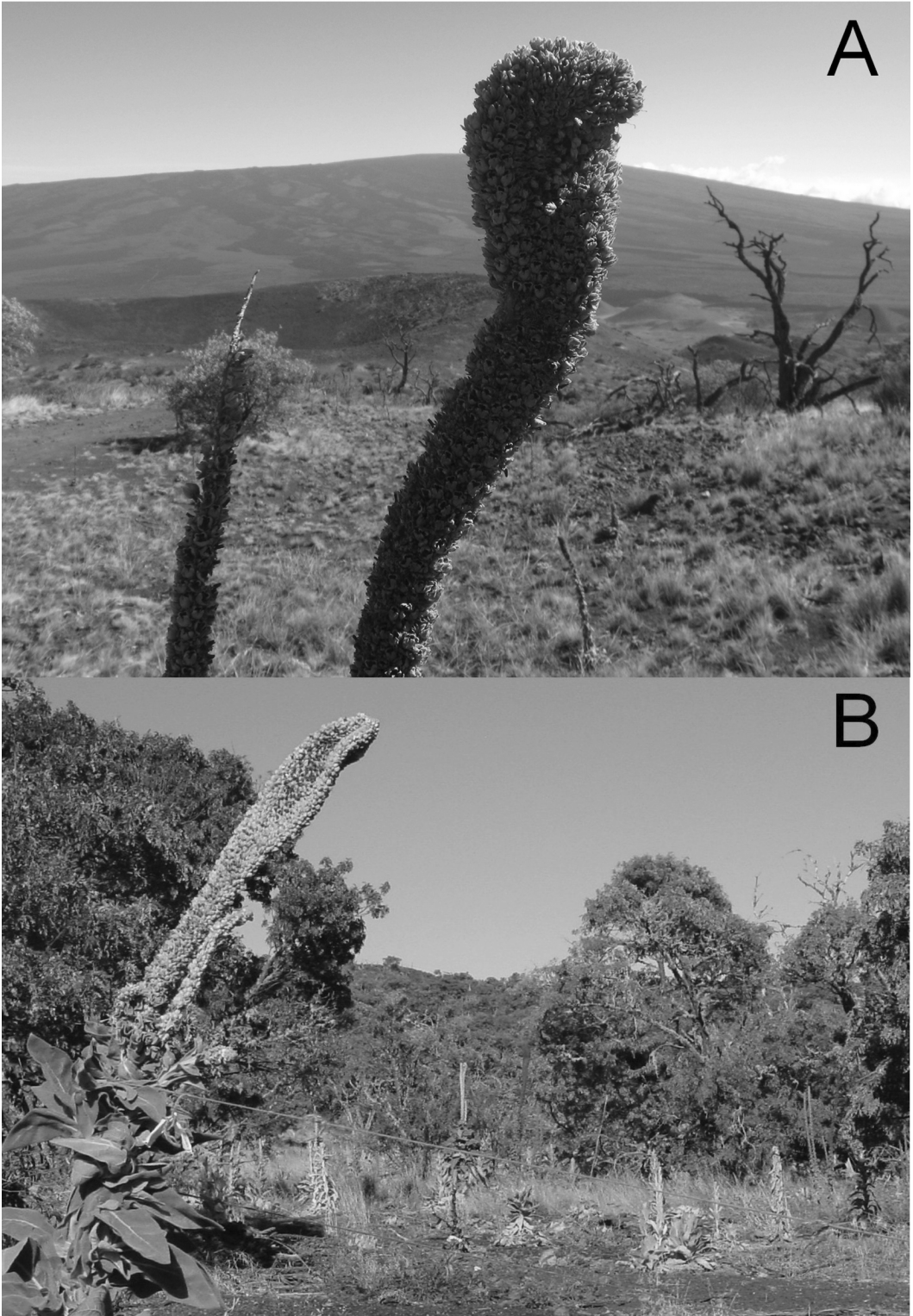
Figure 1. Fasciated *V. thapsus* on the island of Hawai'i: (*A*) pasture vegetation on Mauna Kea, ca. 2,400 m elevation, with normal inflorescence visible in the background; (*B*) natural vegetation on younger lava substrate, Mauna Kea, ca. 2,100 m elevation, with endemic shrubs (*Sophora chrysophylla*) in background.

Hawai'i). The plants were selected in the early flowering stage. On each plant, several unopened flower buds were tagged. The plants were visited early the next morning to perform controlled self-pollinations on the tagged flowers that had opened. Stigmas of freshly opened flowers were inspected to confirm the absence of pollen, which is easily visible, and intrafloral self-pollination was effected using a fine brush. Following self-pollination, a 2 mm mesh was used to enclose the part of the inflorescence containing the self-pollinated flowers to prevent subsequent cross-pollination. At least three flowers were self-pollinated on each plant. The mesh was removed from the plants when capsules had formed. Capsules were collected when they were brown and dry, just before dehiscing. If multiple seed capsules were collected from a plant, they were pooled.

Common Garden

A greenhouse facility at 2,100 m elevation on the island of Hawai'i was used to establish seedlings for the field common garden experiment. Seeds from 31 normal and 29 fasciated plants provided seedlings. Initially, five to 10 seeds from each of the 60 maternal plants were surface-sown on potting soil (Pro-Mix, Premier Horticulture Ltd., Canada) in each of 30 dibble tubes (38 mm diameter, 203 mm long) in a randomized block design. Seedlings in each tube were later randomly thinned to three seedlings at the cotyledon stage and then to one seedling in the four-leaf stage, resulting in 30 replicate seedlings from each of the 60 maternal lines. An automatic misting system watered the seedlings daily, for 10 min every 2 hr during daylight hours. Plants were periodically rotated to ensure even watering.

The site of the field common garden was determined based on the logistics of carrying water to the plants during the initial stages after transplanting. The site also needed to be free from disturbance by military training activities and hunting. Ungulates including cattle, goats, and pigs were present in the area, and some *V. thapsus* plants were observed with broken inflorescences, torn outer leaves, or damaged central leaves among plants in the

rosette stage. Goats were observed to nibble on *V. thapsus* inflorescences, although *V. thapsus* is not a preferred browse plant for ungulates. Because the degree of potential destruction due to ungulates was uncertain, part (25 by 25 m²) of the field garden was enclosed with 1.5 m tall galvanized steel wire fence (hog wire) designed to exclude ungulates, and the remainder was unfenced and exposed to natural damage by ungulates.

In September 2000, near the start of the rainy season, the seedlings were transplanted to the field. Three hundred and twelve seedlings (26 each of normal and fasciated maternal lines; 52 lines × six replicates) were transplanted in the field common garden inside the fence, and 369 seedlings (22 normal and 19 fasciated maternal lines; 41 lines × nine replicates) were transplanted in the adjoining field common garden outside the fence in a complete randomized design. A distance of 1 m was maintained between the seedlings in a row, with 1.5 m between rows. The garden site was relatively open, with only herbaceous vegetation. To avoid unnecessary disturbance, the site was weeded only to remove plants that were overtopping the transplanted rosettes. Inside the fence, two of the six replicates within each line were randomly assigned to a mechanical damage treatment (to simulate ungulate damage). The mechanical damage treatment was applied initially in October 2000 to rosettes that were 10 cm or larger and again 1 month later. If the rosettes that were designated to receive the damage treatment were not 10 cm in October 2000, then the treatment was applied when they reached 10 cm in diameter. All damaged plants were damaged once again when they were first recorded as bolting and flowering. Mechanical damage was applied using pliers to crush and remove the top 2 to 3 cm of the tip of bolting and mature plants, or in rosettes the central leaves were pinched off with pliers. Plants outside the fence were exposed to natural damage, especially from large ungulates. Natural damage on the plants both inside and outside the fence was recorded on all monitoring dates throughout the 3-yr duration of the experiment. The presence or absence of fasciation was noted on each plant throughout

the experiment, and a plant was considered fasciated irrespective of the degree of fasciation. Although fasciation can affect rosettes in *V. thapsus*, fasciation is most often not apparent until the bolting and inflorescence stage; hence in this study, fasciation rates are reported only among plants that survived to the bolting stage or beyond.

Bacterial Isolations

Ten each of normal and fasciated plants were sampled from one site on Mauna Kea at an elevation of 2,485 m. Fresh tissue samples were haphazardly collected in pairs consisting of normal and fasciated plants. First a mature normal plant was selected followed by the closest fasciated individual. The top 15 cm of each inflorescence was cut and collected in a sterile plastic bag with minimal handling. All the bags were stored in a cooler and brought to the laboratory the following day. Pieces of *V. thapsus* tissue from these collected samples were immersed in 0.6% hypochlorite solution for 10 min for surface sterilization and then rinsed with sterile distilled water. Two sets of bacterial isolations were made using these inflorescence samples. For the first set, four small (5 mm) pieces from each inflorescence were placed in petri dishes containing nutrient agar medium with 0.5% yeast extract. In the second set, small pieces of superficial tissue from these plants were dissected and comminuted in sterile water to prepare a slurry, which was vigorously shaken and then used to streak nutrient agar plates. All the inoculated plates were incubated at 25°C. After 7 days the plates were scored for the presence and physical appearance of bacterial colonies. *Rhodococcus fascians*, the recognized causative agent of pathogen-induced fasciation, appears as orange, punctiform colonies on nutrient agar plates incubated at 25°C for 7 days (Odura 1975). In this study, the bacteria were not identified to species, but the bacterial colonies were screened for the color and morphology of *R. fascians*. A third set of plate inoculations was also made using slurry prepared from fasciated and normal plants collected at an elevation of 1,800 m on Mauna Kea.

Field Survey and Inoculations

A field survey was conducted across four sites ranging from 1,900 m to 2,440 m elevation on Mauna Kea to compare the number of capsules produced in normal versus fasciated *V. thapsus* plants. Fifteen each of normal and fasciated plants were randomly selected at each site. To minimize counting errors, a blue permanent marker was used to mark each capsule on the plant during counting.

In February 2003, a field inoculation experiment was conducted at 2,485 m on Mauna Kea at a site that had a high frequency of fasciation. Fasciated tissue samples from 10 fasciated plants were collected and manually mashed together with sterile distilled water using a pestle and mortar in the field. The resultant thick slurry was used to inoculate 10 normal plants in each of three life stages: rosettes, bolting, and flowering. Inoculated plants were paired with controls of similar appearance and size. For rosettes, the leaves in the center were pinched off using pliers and the slurry from fasciated plants was applied using a brush. For bolting and flowering individuals, a superficial cut was made on the side of the stalk using the tip of the pliers, and the top 3 to 4 cm was also mildly crushed with the pliers. The top 5 to 10 cm of the stalk including the wound was coated with the slurry. All control plants were wounded in a similar manner but brushed with sterile distilled water. After 5 months the plants were scored for morphological abnormalities and fasciation.

Statistical Analyses

Common garden results were analyzed by logistic regression (Systat 11, Systat Software Inc., Chicago, Illinois), in which morphology of the garden plants (fasciated or normal) was the dependent variable and parental plant morphology, artificial damage, and natural damage were independent categorical variables. Rates of bacterial isolation from fasciated and normal plants were compared using chi-square exact tests (StatExact 4, Cytel Software Inc., Cambridge, Massachusetts). Outcomes of the field inoculation experiment

were also analyzed by chi-square exact tests. Capsule numbers were compared between normal and fasciated plants across sites using analysis of variance (ANOVA) followed by post hoc contrasts.

<div align="center">RESULTS</div>

Common Garden

A total of 95 plants survived and reached at least the bolting stage by the end of the experiment. This sample size was inadequate for testing between-line variation; therefore, the influence of parental morphology (fasciated or normal) on morphology of progeny was tested by pooling all lines of fasciated or normal plants. Survival rate did not differ by parental plant morphology ($\chi^2 = 0.20$, $P = .33$).

Overall, 31% of plants developed fasciation. There was no statistical difference in the fasciation rate inside and outside the fence (t-ratio $= -1.56$, $P = .12$); therefore, all plants were combined for logistic regression (Table 1). Morphology of the parental plant was not statistically linked to morphology of progeny in the common garden ($P = .26$, Table 1, Figure 2). Although the regression coefficient for parental morphology was positive, it had a large standard error (Table 1).

Thirty-seven percent of the analyzed plants were in the artificial damage treatment. Contrary to our initial prediction, artificial damage was associated with lower rates of fasciation ($P = .023$, Table 1, Figure 2). The 95% confidence interval of the odds ratio from logistic regression indicates that damaged plants were between 1.3 and 32 times less likely to become fasciated. Artificial damage did not interact with parental morphology (Table 1), indicating that the inhibitory effects of artificial damage on fasciation did not depend on morphology of the parental plant.

In total, 13% of plants were naturally damaged. Damage ranged from apparently chewed leaves to a broken stalk. Natural damage was not statistically associated with fasciation in the logistic regression ($P = .23$, Table 1); among the seven plants that had natural damage without artificial damage, none developed fasciation (Figure 2), whereas the overall fasciation rate for undamaged plants was 48%. A chi-square exact test reveals that these rates are statistically different ($\chi^2 = 5.84$, df $= 1$, $P = .035$ without midpoint correction, $P = .027$ with midpoint correction) and consistent with the inhibitory effects observed in artificially damaged plants. Logistic regression combining artificial and natural damage identifies a significantly lower probability of fasciation associated with damage ($P = .009$) but no effect of parental morphology ($P = .18$) and no interaction between parental morphology and damage ($P = .86$).

Bacteria Isolations

None of the bacterial colonies isolated from fasciated or normal *V. thapsus* resembled the colony phenotype of *Rhodococcus fascians*. Isolations made directly from dissected plant

<div align="center">TABLE 1</div>

Logistic Regression Testing Influence of Parental Morphology (Fasciated or Normal), Natural Damage (Present or Absent), and Artificial Damage Treatment (Yes or No) on Morphology of Plants (Fasciated or Normal) in a Field Common Garden

Factor	Estimate	S.E.	t-Ratio	P-Value
Parental morphology	0.615	0.550	1.12	.26
Natural damage	−1.024	0.856	−1.20	.23
Artificial damage	−1.857	0.820	−2.27	.023
Artificial damage × parental morphology	0.211	1.206	0.18	.86
Constant	−0.452	0.356	−1.27	.20

Note: Log Likelihood of the model is −51.21 versus −58.45 for the constant only model ($\chi^2 = 14.48$, df $= 3$, $P = .002$).
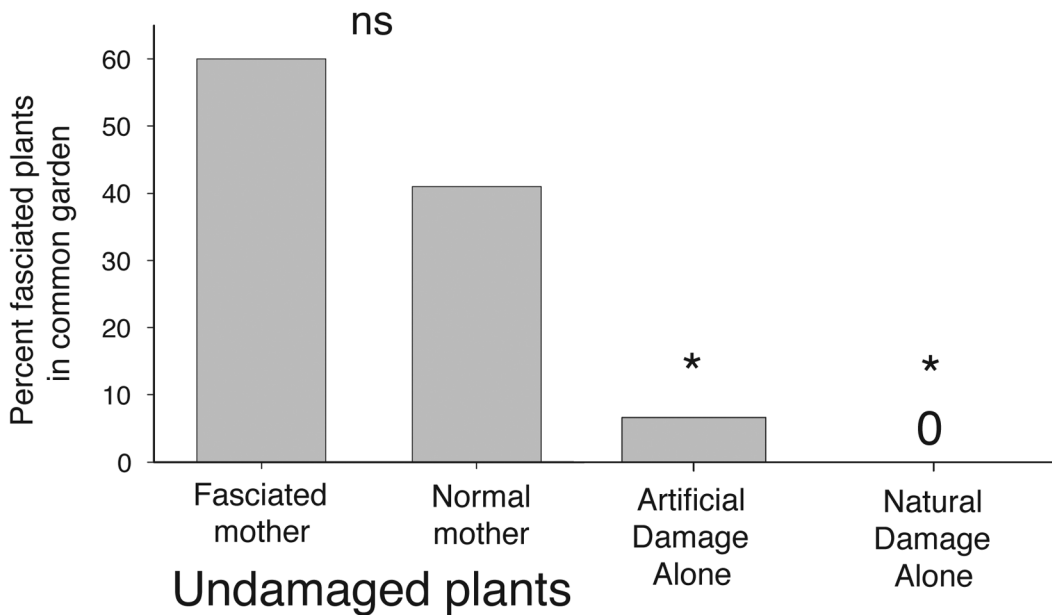
FIGURE 2. Fasciation rates in undamaged plants versus plants exposed to one type of damage. ns, no statistical difference between progeny of fasciated and normal mothers; *, significant difference between damaged and undamaged plants ($P < .05$).

tissue from 2,485 m elevation on Mauna Kea showed the presence of only white and yellow bacterial colonies, and there were no significant differences in the frequency of occurrence of these colonies between normal and fasciated plants (Figure 3). Isolations derived from the slurry revealed five different colony morphologies, including an orange morph, but rates of isolation did not differ between fasciated and normal plants (Figure 3). The same five colony morphs were also isolated from plants collected at the 1,800 m site, but again there were no differences in isolation rates between normal and fasciated plants (data not shown). For plants collected at 1,800 m, orange colonies were cultured from slurries derived from 20% of the fasciated plants and 25% of the normal plants (no statistical difference), and the orange colonies were not punctiform, as would be expected for *R. fascians*.

Field Survey and Inoculations

Across all four sites, fasciated plants averaged more than twice the number of capsules and seeds as normal plants, and within each site, fasciated plants always averaged more capsules (Figure 4). Among the field-inoculated plants, none developed fasciation irrespective of the treatment (slurry or water control) and growth stage (Table 2). The damaged stem and leaf tissue immediately adjacent to the site of wounding inoculation sometimes appeared crinkled, but there were no differences between treatment and controls in the rate or extent of localized leaf deformation (Table 2). Irrespective of treatment, there was 100% survival among rosettes and there were no significant differences between the slurry treatment and controls in the survival of plants at the bolting and flowering stage (Table 2).
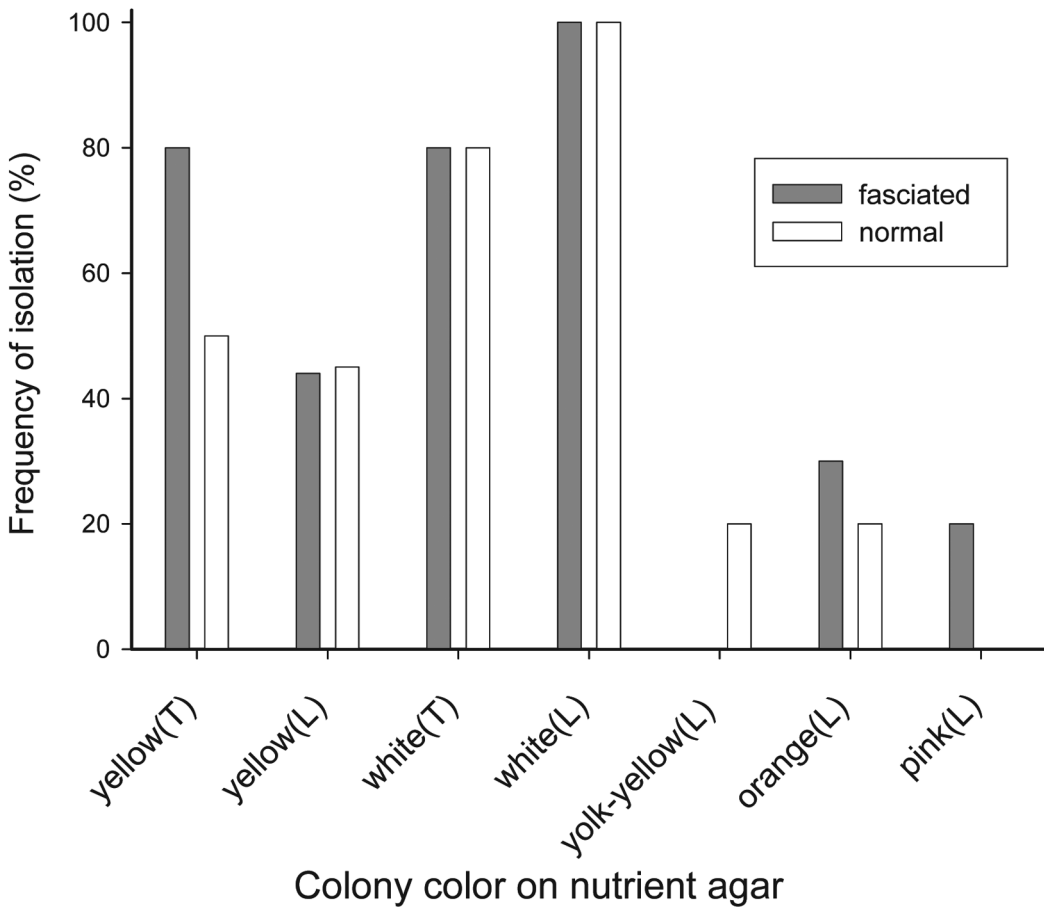
Figure 3. Frequency of occurrence of different-colored colonies from normal and fasciated plants collected at 2,485 m on Mauna Kea (T, cultured directly from tissue; L, cultured from the tissue slurry). *Rhodococcus fascians*, a pathogenic bacteria that can cause fasciation, is expected to form orange colonies. There were no significant differences in isolation rates between normal and fasciated plants.

DISCUSSION

Does Propensity toward Fasciation Have a Genetic Basis?

Although significantly higher capsule and seed production in fasciated *V. thapsus* suggests that fasciation could be ecologically important and help promote the success of this invasive weed, fasciation does not appear to be heritable. If fasciation in *V. thapsus* were controlled by a single recessive or dominant gene, then the self-pollinated fasciated par-ents would have produced progeny that were 100% fasciated (if recessive) or either 75% or 100% fasciated (if dominant), and fasciation should have occurred at a much lower rate (0% if fasciation was dominant, and 25% if fasciation was recessive) in progeny of normal parents. In this study, only about half of un-damaged progeny of fasciated parents were fasciated, and this rate was similar among progeny derived from selfed normal plants. These results indicate that, unlike in some agricultural plants such as soybean (*Glycine max*), mung bean (*Vigna radiata*), lentil (*Lens*
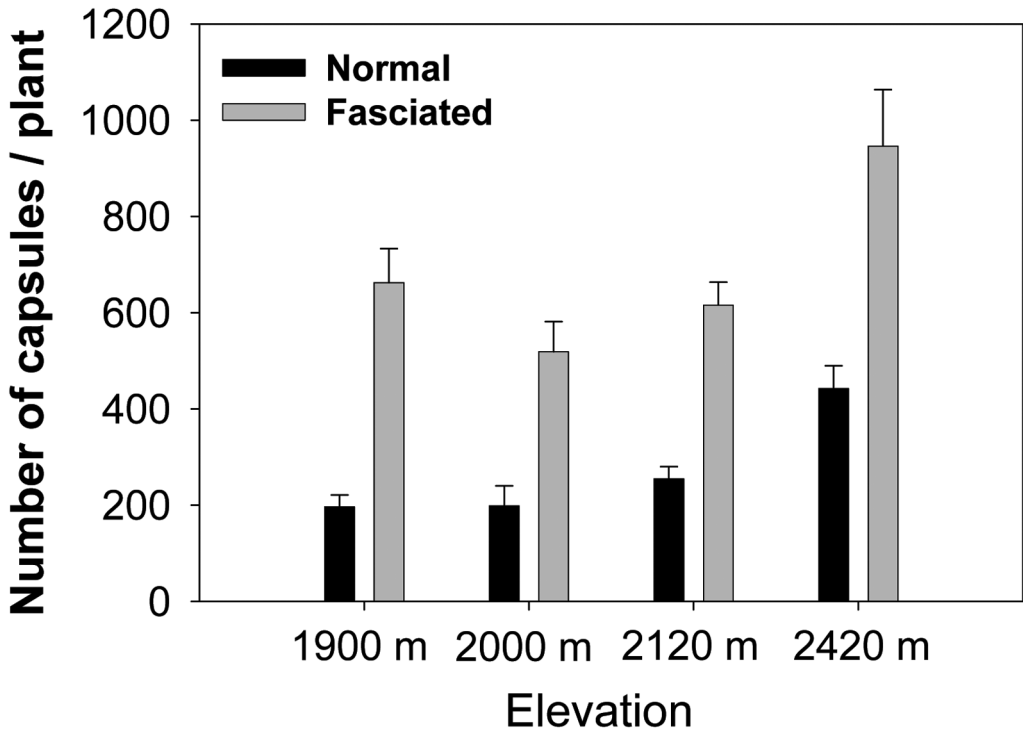
FIGURE 4. Comparison of capsule production in normal versus fasciated *V. thapsus* across four sites ranging from 1,900 m to 2,440 m on the island of Hawai'i. Fifteen fasciated and 15 normal plants were measured at each site. All differences are statistically significant ($P < .05$, ANOVA followed by post hoc contrasts). Error bars indicate 1 SE.

TABLE 2

Survival, Development of Deformed Leaves, and Fasciation among Field Plants Inoculated with a Slurry Extracted from Fasciated Plant Tissues

Stage	% of Plants That Survived		% of Plants with Deformed Leaves at Point of Injury		% Fasciated	
	Control	Inoculated	Control	Inoculated	Control	Inoculated
Rosette	100	100	60	60	0	0
Bolting	50	70 ns	10	10	0	0
Flowering	20	50 ns	0	0	0	0

Note: ns, no statistical difference, $P > .05$.

culinaris), chickpea (*Cicer arietinum*), and red beet (*Beta vulgaris*) (Albertsen et al. 1983, Dwivedi and Singh 1990, Tyagi and Gupta 1991, Knights 1993, Goldman 1998), the fasciated phenotype in *V. thapsus* does not follow a simple biallelic pattern of inheritance. Furthermore, the lack of difference in fasciation rate between progeny of fasciated and normal plants provides no evidence at all for heritability.

Although our results indicate that fasciation in *V. thapsus* in Hawai'i probably does not have a simple genetic basis, a multiallelic genetic basis for fasciation has been reported in several plant species (Gottschalk and Hussein 1977, Eenink and Garretsen 1980, Albertsen et al. 1983), and fasciation may develop as a result of gene by environment interaction (Robinson 1987). It was beyond the scope of this study to investigate the possibility of such complex scenarios in *V. thapsus*.

Does Damage Predispose Plants to Fasciation?

If physical damage caused fasciation, then damaged plants should have had higher rates of fasciation, but this study found the opposite. Feral goats were observed around the experimental garden several times during the course of the study (S.A., pers. obs.). Nevertheless, natural damage rates were low (around 10%), and this damage did not promote fasciation.

In a study in North America (Naber and Aarssen 1998), no fasciations were reported in *V. thapsus* following experimental removal of the apical meristems. In *Lilium henryi*, wounding of healthy plants in various stages of development by either damaging the growing point, decapitation, or defoliation also did not induce fasciation (Stumm-Tegethoff and Linskens 1985). The evidence that mechanical damage causes fasciation is mainly anecdotal (White 1943, 1948).

Insects have been reported to cause fasciation in some plants (Knox 1908, Binggelli 1990). We conducted field surveys of *V. thapsus* on the island of Hawai'i from sea level to 3,000 m, but little or no evidence of insect damage was observed. A few plants in a population at Pu'uwa'awa'a Natural Area Reserve (545 m) showed evidence of seed predation; however, no insects were observed on those plants. The seed predation occurred after capsules had matured and therefore cannot be linked to fasciation. Dissection of fasciated plants also did not reveal any activity of sucking or internally feeding insects or nematodes. Hence it is unlikely that fasciation in *V. thapsus* is caused or mediated by insects or nematodes.

Because the frequency of fasciation was initially reported to increase with elevation in Hawai'i, Juvik and Juvik (1992) proposed that diurnal frost damage at high elevation might cause fasciation. However, a high frequency of fasciation (50%) in populations located at persistently warm sites near sea level (50 m) has subsequently been observed (S.A., unpubl. data), so factors other than frost must be involved.

Is Fasciation in V. thapsus *Induced by Bacteria?*

In this study, none of the isolated bacterial colonies had cultural characteristics similar to those of *Rhodococcus fascians*. However, because it is sometimes difficult to isolate bacteria from infected tissue, the isolation results alone provide rather weak evidence against the bacteria hypothesis. In contrast, the failure of all field-inoculated plants to develop fasciation provides stronger evidence that fasciation in *V. thapsus* is not caused by a pathogen. The bacteria can be spread easily from infected plants, seeds, and soil on hands or tools (Putnam and Miller 2007). Two forms of *R. fascians* bacteria are known. One of them is epiphytic, only colonizing the plant surface, affecting shoot meristem formation; the other form is endophytic, and this form is required for symptom maintenance (Goethals et al. 2001, Vereecke et al. 2002). Plants infected with *R. fascians* are often substantially debilitated by the pathogen. In *Chrysanthemum maximum* and *Pisum sativum*, plants infected with *R. fascians* show hypertrophied shoots, and they are stunted and appear weak compared with noninfected plants (Odura 1975). Infection at the seedling stage may result in complete inhibition of seedling growth, including inhibition of root growth and thickening of the hypocotyls (Vereecke et al. 2000). The secondary symptoms accompanying hypertrophied shoot formation are stunted growth, reduction in flower size, and reduced yield (Odura 1975). Contrary to these debilitating symptoms, in *V. thapsus* the fasciated plants in the field appear to be more robust, taller, and larger than normal plants, and they produce more capsules. Greater yield in fasciated plants has not been reported

in bacteria-associated fasciation, but it is sometimes seen when fasciation is caused by other factors (e.g., Tyagi and Gupta 1991). Potential fitness trade-offs associated with higher yield in nonbacterial fasciations have not been investigated.

Potential Environmental Factors

Detailed breeding experiments by DeVries (1908) and White (1948) showed that in some plants fasciation was expressed only under certain environmental conditions. Muncie and Patel (1930) suggested that in sweet peas, fasciation was induced by overcrowding of their roots. In a separate experiment they further showed that wounding by puncturing the base of stems with a sterile needle, combined with crowding, led to fasciation rates of 28% in comparison to 17% in undamaged controls ($\chi^2 = 5.90$, df = 1, $P = .015$). Muncie and Patel (1930) concluded that abnormal growing conditions induce fasciation in sweet peas.

Contrary to Muncie and Patel's (1930) findings on peas, increased spacing and favorable growing season increased fasciation rates in flax (*Linum usitatissimum*) (Shibuya 1939). Many early researchers speculated that high levels of nutrition induce fasciation, but none provided any experimental evidence for this (Hus 1908, Knox 1908, White 1943, 1948). Several other explanations such as changing the photoperiod and increased nutrition with water shortage have been speculated to induce fasciation (Binggelli 1990). Zinc deficiency has also been reported to induce fasciation in plants (Rance et al. 1982) while also causing a characteristic suite of debilitating symptoms that are not observed in *V. thapsus*.

Coarse-scale environmental factors in Hawai'i, such as a year-round growing season and high ultraviolet radiation, may promote fasciation, with susceptibility modified by local factors such as disturbance, exposure, or perhaps genetics. A localized contributing factor is supported by the fact that normal and fasciated plants commonly occur in close proximity (<1 m) in the field. The ecological or evolutionary significance of fasciation in natural populations has rarely been examined. In addition to producing more capsules, fasci-

ated *V. thapsus* develop a degree of stem woodiness that is not seen in normal plants (Juvik and Juvik 1992). Woodiness may be a by-product of longer life span, which may benefit island colonizers by allowing more opportunities for cross-pollination and escape from detrimental inbreeding (Böhle et al. 1996), but a reproductive advantage associated with greater investment in reproduction (allowed for by woody stalks) would also greatly benefit a colonist during island invasion. This is especially true when risks of juvenile mortality are relatively low, as was true in high-elevation populations in Hawai'i where *V. thapsus* is unusually long-lived (Ansari and Daehler 2010). Although we were unable to demonstrate a simple genetic basis for fasciation in invading *V. thapsus*, our statistical power to detect small degrees of heritability was low, and we did not test for gene-by-environment interactions. If fasciation does have an element of heritability, then we expect to see a long-term pattern of increasing gigantism and adaptive radiation in *V. thapsus* as it invades Hawai'i, as has been observed in numerous other island colonizations by rosette plants over evolutionary time (Carlquist 1974).

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