Plant water stress and gall formation (Cecidomyiidae: *Asphondylia* spp.) on creosote bush

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ABSTRACT. 1. Populations of creosote bush (*Larrea tridentata* (DC) Coville), were studied in Arizona to determine whether associated gall-formers (Cecidomyiidae: *Asphondylia* spp.) were more abundant on water-stressed or nonstressed plants. Gall densities were measured along a steep elevational gradient that extended from mesic, higher elevations to lower elevations in the desert; and in the Grand Canyon where severely water-stressed and relatively unstressed plants occurred adjacently. At the Grand Canyon site, the responses of creosote bush to water stress were also studied.

2. The number and densities of *Asphondylia* species increased both at lower elevations and locally on water-stressed plants in the Grand Canyon, indicating that climatic and local conditions influence gallformer abundance in the same way.

3. Five of the eight *Asphondylia* species studied at the Grand Canyon site were more abundant on stressed plants, two species were more abundant on nonstressed plants and one species showed no preference for either plant type.

4. Densities of most species on stressed plants were positively correlated with the number of meristematic terminals per branch, which were more numerous on stressed plants, due to a bushier architecture. Flower gall-formers were more abundant on nonstressed plants, which produced more flowers. Gall densities did not correlate with chemistry measurements, although these parameters also varied with level of stress.

5. These results suggest that gallforming species respond variably to plant stress, even within a closely-related lineage, and that there are effects of stress on plants, including architectural changes, that may be more important to herbivores than biochemical effects emphasized by White (1984) and others.

Key words. *Larrea tridentata*, *Asphondylia*, Cecidomyiidae, creosote bush, plant stress, gallforming, herbivory.

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Introduction

While the ecological literature has emphasized the importance of plant stress in improving plant quality for herbivorous insects, there is evidence that many gallforming insects are dependent upon vigorous plant growth (e.g. Washburn & Cornell, 1981; Frankie & Morgan, 1984; Craig *et al.*, 1986; Abrahamson & Weis, 1987; Waring & Price, 1988; Weis *et al.*, 1988; Price, 1989). Such opposing patterns reflect a need for alternate hypotheses concerning plant–herbivore interactions, and the relationship of gallforming insects with plant vigour, which we term the 'vigour hypothesis', may be useful. The predictions of these two hypotheses differ, with stress-related changes in nutritional and defensive chemistry forming the basis for the stress hypothesis (e.g. White, 1984; Rhoades, 1983), while for gallformers, the vigour hypothesis predicts a need for developing tissue for gall formation and perhaps for acquisition of nutrients (Abrahamson & Weis, 1987).

Numerous studies have shown that gallforming insects have life cycles that are highly synchronized with patterns of host plant growth (Whitham, 1978; Brewer & Skuhravy, 1980; Weis *et al.*, 1988). Univoltine species are typically associated with plants that exhibit discontinuous growth, whereas multivoltine species often associate with plant species that grow vigorously throughout the year (Abrahamson & Weis, 1987). Craig *et al.* (1986) and Waring & Price (1988) found that vigorously growing willow shoots were more susceptible to gallformers and offered improved survivorship. Washburn & Cornell (1981) found that as plant parts aged and vigorous growth declined, the parts became less susceptible to gallformers.

If plant vigour is a major determinant of gallforming success, then environmental factors which can retard growth, such as water stress, should restrict the distribution of gallformers within their host plants' range temporally or spatially. Such a relationship was found for the *Salix lasiolepis* Bentham – *Euura lasiolepis* Smith (willow-sawfly) system (Price & Clancy, 1986). Reduced winter precipitation in some years strongly reduced sawfly survivorship and galls were less abundant on plants without access to a permanent water source.

The potential generality of gallforming dependence on plant vigour prompted us to study

the desert-dwelling shrub, creosote bush (*Larrea tridentata* (DC) Coville), and its associated gallforming community (Cecidomyiidae: *Asphondylia* spp.) to determine the role of plant vigour in this association. If these gallformers are dependent on vigorously growing plants, they should be rare or absent on water-stressed plants. We addressed this prediction by measuring *Asphondylia* species richness and densities, (1) along a steep elevational gradient that ended in harsh desert conditions, and (2) locally where stressed and nonstressed plants were both available for colonization. This also offered the opportunity to determine how uniform the responses of a closely-related gallforming lineage are to plant stress.

Methods and Materials

Natural history. *Larrea tridentata* is the dominant plant species throughout most of the southwestern deserts, and is one of the most drought tolerant species occurring there (Barbour, 1968). Despite its tolerance of water stress, it can exhibit many traits characteristic of stressed plants. In a water stress experiment with creosote bush seedlings, the leaf tissue of water-stressed plants had significantly greater concentrations of many amino acids than nonstressed plants (Saunier *et al.*, 1968). Greater concentrations of amino acids and other solutes have been found in leaf tissue of water-stressed plants in many plant species (White, 1984) and may aid in osmoregulation (Morgan, 1984). The level of sexual reproduction in creosote bush is positively tied to water availability (Barbour *et al.*, 1977) and plant height can vary by as much as a metre, depending on water availability (Cunningham & Burk, 1973). Cunningham & Burk (1973) and Cunningham *et al.* (1979) showed that creosote bushes growing in shallow soils were more water-stressed and produced less above-ground biomass than plants growing in deeper soils. In Arizona it is common to see tall, luxuriant plants growing along roads or washes, while plants 10 m away can be stunted and severely water-stressed (Waring, 1987). Such conditions are ideal for considering the response of associated herbivores to plant stress, because stressed and nonstressed plants often occur within the herbivore's cruising range.

The term 'stress' is used here to characterize

water deficiency in plants, which is reflected in reduced growth and reproduction, as well as biochemical changes (Boyer, 1982; White, 1984). The term 'nonstressed' is used here in a relative sense, referring to plants which are experiencing smaller water deficits than 'stressed' plants. Xylem water potential and other plant traits were measured to establish these relative designations.

Creosote bush's most consistent herbivores include a monophyletic group of sixteen species of cecidomyiids (Cecidomyiidae: *Asphondylia* spp.) that form galls on its leaves, stems and flowers through most of its North American range (Waring, 1987). *Asphondylia auripila* Felt was described by Felt (1965) and the other fifteen species are being described (Gagné & Waring, in preparation). In this paper, the species are referred to by number.

Elevational study. This study examined whether *Asphondylia* species richness and densities decline at lower, more arid elevations along a steep elevational gradient. Twenty-three sites through creosote bush's range in Arizona were sampled over a distance of 400 km, and 280–1170 m in elevation, in August 1985. These sites spanned a gradient from mesic, high elevation to xeric, low elevation conditions in the Sonoran Desert. The amount of precipitation in Arizona increases 12.5 cm per 305 m elevation (Lowe, 1964). All sites were located away from roads and drainages and all had southern exposure. Ten plants were randomly selected per site and two branches in the upper canopy per plant were collected for determining numbers of galls and species. Elevation was determined at each site and was adjusted for latitude (4° latitude per 305 m; Lowe, 1964).

Plant stress study. This study examined the effects of water stress on creosote bush and associated gallforming *Asphondylia* species at a site where both water-stressed and nonstressed plants occurred. The work was done at Colorado River mile 198 in Grand Canyon National Park, Arizona. This region occurs at 430 m above sea level at 36.1° latitude, at the eastern edge of the Mojave Desert. At this site, creosote bush grew on steep, shallow soil talus slopes (slope 29°) along the canyon wall (Talus site) and on an adjacent flat, deep soil bench (slope 0°) (Flats site). The close proximity of both plant types made them both available to gallforming *Asphondylia* species for colonization. Creosote bush grew in stunted form along talus slopes throughout the lower Grand Canyon, with occasional more luxuriant stands occurring in the rarer deeper soil habitats, such as the Flats site.

We measured a number of plant traits to verify apparent differences in water stress. Pre-dawn xylem water potential of ten Talus and ten Flats plants was measured with a Scholander Pressure Chamber® between 05.00 and 06.00 MST on 20 August 1984. During August, eight morphological and physiological plant measurements were taken: height (m); reproduction (no. of seeds per gram (g) of branch); growth rate (stems were marked and growth recorded (four branches per plant were measured and averaged) between March and August 1985); number of meristematic terminals per g branch (four branches per plant); per cent phenolic resins per leaf; total phenols per mg leaf tissue; per cent total soluble proteins; and per cent total Kjeldahl N.

Biochemical measurements of leaves were evaluated as additional indicators of plant stress (*sensu* White, 1984) and to determine if gallformer densities were correlated with them. To determine percentage resin volume, freeze-dried leaves were weighed on a Cahn® electrobalance, dipped into three successive ether baths, dried and reweighed, with the difference in mass due to resin loss (Rhoades, 1977). Kjeldahl nitrogen analysis followed that of Parkinson & Allen (1975). Per cent total soluble protein analysis used the BioRad® assay (Bradford, 1976) and total phenol analysis followed the Folin Denis assay using the methods of Zucker (1982). These measures determined relative differences in resin volume and protein, N and phenol concentrations among plants.

Densities of eight *Asphondylia* spp. were measured on Talus plants (stressed) and Flats plants (nonstressed) for 3 years. The *Asphondylia* species form galls on four different plant parts: on upper leaf surfaces (spp. 1–3), on lower leaf surfaces (spp. 4–6), on stems (sp. 11), and in flowers (sp. 10). In August 1984 and 1985 and in September 1986 two branches from each of twenty plants (1984), sixteen plants (1985) and thirty plants (1986) from the Talus population and the Flats population were randomly collected. Flower galls were studied in 1988. In the laboratory galls were counted per branch; the branches were oven dried, foliage was weighed, and green meristematic terminals counted.

Pupal weight and survivorship on stressed and

nonstressed plants could be measured for *Asphondylia* sp. 11 only. In September 1986, 50 galls/plant for 8 plants/site were sampled and survivorship and mortality were determined (fly pupa or emergence hole=survived; parasitized; fly dead or absent=unexplained mortality, possibly due to plant quality). Sexed pupae and galls were weighed.

Statistical analysis included linear and non-linear regression to correlate densities of galls with elevation and latitude (Nie *et al.*, 1970). Multivariate analysis of variance (MANOVA) was used to compare densities of galls at the Grand Canyon site on stressed and nonstressed plants among species and years (Hull & Nie, 1981). The Shannon diversity index (H') was used to compare gall community diversity on stressed and nonstressed plants (Zar, 1984), with the higher H' reflecting a community in which species are more numerous and more evenly represented. H' values were compared with a two-sample *t*-test (Hutcheson, 1970). Two

canonical correlation analyses (Nie *et al.*, 1970) tested for correlations between species densities and (1) plant chemistry and (2) plant growth and number of meristematic terminals. ANOVA was used to compare pupal mass and plant trait patterns at the Grand Canyon site, and per cent mortality of *Asphondylia* sp. 11 on stressed and nonstressed plants was arcsin-square root transformed (Zar, 1984) and analysed with a pooled *t*-test (Nie *et al.*, 1970).

Results

Elevational change and gall densities

Asphondylia species numbers and densities were greater at lower than at higher elevations, although both declined somewhat at the lowest elevations (Fig. 1), implying an elevational threshold for some species. Up to eleven species were encountered at lower elevations, whereas
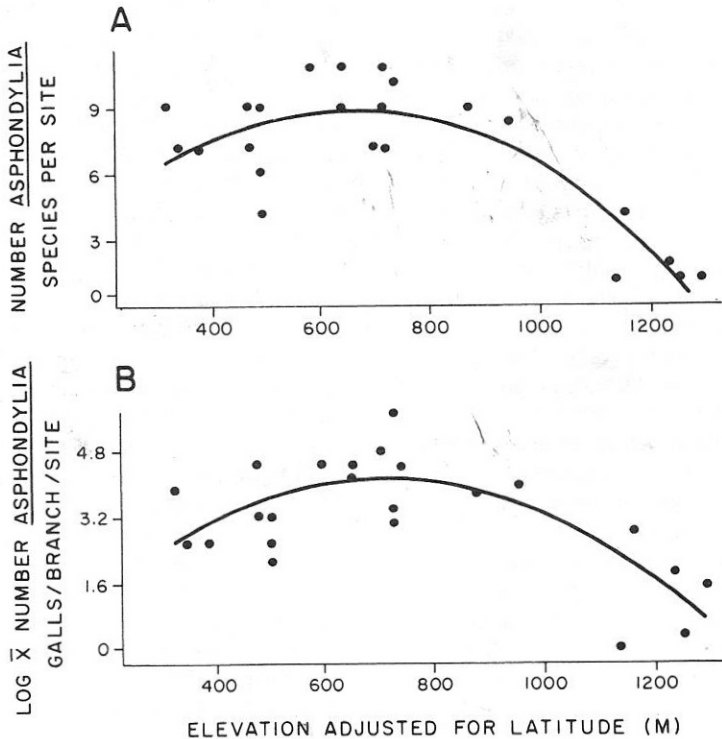

FIG. 1. (A) The relationship between *Asphondylia* species richness and elevation adjusted for latitude ($r^2=0.70$, $y=-201+10.2x-0.124x^2$, $P<0.001$, $n=23$). (B) The relationship between the log density of *Asphondylia* galls and elevation adjusted for latitude ($r^2=0.52$, $\log_{10} y=-93.7+4.66x-0.058x^2$, $P<0.005$, $n=23$).

the fewest species occurred at the higher elevations. Densities of *Asphondylia* showed the same patterns: densities were lowest at the highest elevations, increased at lower elevations and declined somewhat at the lowest elevations (Fig. 1). These results indicate that the distributions of *Asphondylia* species on creosote bush are strongly correlated with decreased elevation and latitude, which coincide with increased environmental stress. However, there appears to be an environmental limit below which the distributions of these species begin to decline.

The large densities of *Asphondylia* at the Grand Canyon site (see below) fit this line well, although this site is more than a full latitudinal degree north of all other sites and separated from them by mountainous terrain. When the elevation and latitude of this site are taken into account, it lies at 725 m (Fig. 1), along with other high density sites. This seems to reaffirm that both elevation and latitude influence gall densities.

Creosote bush and water stress

At the Grand Canyon site there were major morphological and physiological differences between creosote bushes on the Flats and the Talus (Table 1), probably due to microsite differences

in soil depth and slope. Scholander pressure chamber measurements and seasonal growth patterns showed that plants on the Talus were significantly more water-stressed than those on the Flats (Table 1). The plants on the Talus grew less in the spring (March–May) than did plants on the Flats (Table 1), suggesting that shallower Talus soils were unable to capture as much moisture from springtime precipitation. There was no stem growth in either of the two plant groups during the summer (June–July), due to a lack of precipitation (Table 1). Branch dieback resulted in negative growth values (Table 1). This pattern of growth is common for creosote bush in the Southwest (Lightfoot & Whitford, 1987; Cunningham *et al.*, 1979).

Creosote bushes on Talus and Flats sites also differed architecturally and biochemically (Table 1). Talus plants were significantly shorter, with more lateral and diffuse growth than non-stressed plants, as determined by the number of meristematic terminals on branches (Table 1). Stressed Talus plants supported a low diffuse canopy to carry on photosynthesis, while plants on the Flats were tall, investing more in woody apical growth.

The stressed talus plants produced a larger volume of phenolic resins on leaf surfaces. They also had greater concentrations of total proteins

TABLE 1. Mean measurements of plant traits of nonstressed (Flats) and water-stressed (Talus) creosote bushes at Parashant Canyon, Grand Canyon.

	Flats (SE)	Talus (SE)	*P*
Morphological			
Plant height (m)	2.21 (0.13)	1.47 (0.90)	<0.005
Reproduction (seeds/branch)	66.50 (1.36)	7.50 (1.07)	<0.005
No. meristematic terminals/g foliage			
1985	12.81 (1.67)	28.94 (3.38)	<0.005
1986	12.65 (1.40)	19.89 (1.35)	<0.005
Physiological			
Tissue water potential/(−Bars)	35.20 (1.47)	48.80 (1.82)	<0.005
Branch growth (cm)			
March–May	2.21 (0.36)	0.65 (0.28)	<0.010
June–August	−0.13 (0.20)	−0.17 (0.20)	nsd
Per cent phenolic leaf resin (of total weight)	23.00 (1.91)	34.00 (1.67)	<0.005
Total leaf phenols (absorbance/mg/ng 595)	0.12 (0.00)	0.09 (0.00)	<0.010
Per cent total leaf proteins	19.10 (1.24)	22.30 (0.71)	<0.050
Per cent total leaf Kjeldahl N	2.38 (0.15)	2.90 (0.07)	<0.005

and total Kjeldahl N and smaller total phenol concentrations of leaf tissue (Table 1), which are common responses in water-stressed plants (White, 1984).

Gallformer responses to plant stress

Five of eight *Asphondylia* species studied were more abundant on the water-stressed Talus plants than on the vigorously growing plants on the Flats (Table 2). Mean gallformer densities were 3 times greater on stressed Talus plants. Two of the other three *Asphondylia* species (leaf gallformer 8 and flower gallformer 10) were significantly more abundant on Flats plants, while the third (gallformer 2) was equally distributed across the two plant types (Table 2).

The gall community on the Flats was significantly more diverse ($H'=1.639$) than that on the stressed Talus plants ($H'=1.299$) (two sample $t=2.897$, $P<0.005$, df=132), due to greater densities of some *Asphondylia* species on stressed Talus plants (Flats $J'=0.529$; Talus $J'=0.362$), although all species were found on both plant types.

Although many features of plant morphology and physiology differed between stressed and nonstressed plants, the densities of *Asphondylia* species correlated strongly only with morphological features (Table 3). There were significant correlations between *Asphondylia* species densities and creosote bush growth and number of meristematic terminals (Table 3). Simple regressions indicated that the densities of four *Asphondylia* species were significantly positively correlated with the number of meristematic terminals per branch (Table 3; for all four species: $r^2=0.61$, $y=0.21+1.04x$, $n=16$, $P<0.005$). The densities of another species, *A.* sp. 2, were significantly correlated with plant growth (Table 3), although this species was not more abundant on either plant type. Densities of the flower gallformer, *A.* sp. 10, were strongly correlated with the number of flowers and seeds per branch (Table 3), and this species was significantly more abundant on nonstressed plants, which produced significantly more flowers and seeds (Table 1).

Stressed plants were of comparable or slightly better quality as resources for *Asphondylia* sp. 11. There was a trend of decreased mortality in *A.* sp. 11 on stressed plants (mean per cent mortality, Flats: 21.2, SE=5.4; Talus: 8.5, SE=2.8; pooled $t=1.64$, $P=0.12$, $n=16$). Pupal weights and gall weights of *A.* sp. 11 did not differ significantly on the two plant types, although there was a trend of larger female galls on water-stressed plants (Table 4). Per cent parasitism of *Asphondylia* sp. 11 was 20 per cent higher on stressed plants, perhaps due to higher gall densities (mean per cent parasitism, Flats: 37.0, SE=5.0; Talus: 58.0, SE=4.3).

TABLE 2. Mean densities (per 10 g foliage) of *Asphondylia* species on nonstressed (Flats) and water-stressed (Talus) creosote bushes from 1984 to 1986 at Parashant Canyon, Grand Canyon (*$P<0.05$, **$P<0.005$).

Asphondylia species	Densities by site, mean (SE)		Probability		
	Flats	Talus	Site	Year	Interaction
Leaf gallformers					
1	7.0 (0.9)	27.0 (7.2)	**	**	**
2	6.6 (1.6)	6.6 (2.9)	ns	*	ns
3	10.4 (1.7)	28.9 (7.0)	**	*	ns
6	2.7 (0.7)	6.2 (1.6)	**	*	*
7	30.7 (6.6)	144.1 (24.0)	**	ns	ns
8	4.4 (1.3)	2.2 (1.1)	*	*	ns
Flower gallformer					
10	3.0 (1.1)	0.1 (0.0)	**	*	ns
Stem gallformer					
11	7.1 (1.6)	30.5 (3.4)	**	ns	ns
Total	79.0 (10.0)	246.5 (35.0)	**	**	**