The distribution of flower galls caused by *Anguina amsinckiae* on the weed, common fiddleneck, *Amsinckia intermedia*

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SUMMARY

The distribution of flower galls caused by *Anguina amsinckiae* (Steiner & Scott, 1934) Thorne, 1961 on the host plant *Amsinckia intermedia* Fischer & Meyer (Borraginaceae) is described. Galls were exclusively associated with floral tissues, and were restricted to the five uppermost inflorescences of the host plants. Approximately 93 % of total galls were located in the two uppermost inflorescences. Within a given inflorescence, galls were restricted to the six basal flowers with approximately 82 % of the galls being distributed between the most basal flower and the flower directly above it. This gall distribution suggests that nematode attack occurs during the host's early flowering stages, and that the pattern observed may be a function of moisture availability to the nematode during infection. Plant growth regulators are suggested as a possible means of enhancing the onset and degree of galling.

RÉSUMÉ

La répartition des galles florales causées par Anguina amsinckiae *sur la plante* Amsinckia intermedia

La répartition des galles florales dues à *Anguina amsinckiae* (Steiner & Scott, 1934) Thorne, 1961 sur la plante *Amsinckia intermedia* Fischer & Meyer (Borraginacées) est décrite. Ces galles sont associées aux tissus floraux et restreintes aux cinq inflorescences les plus élevées. Environ 93 % des galles sont situées sur les deux inflorescences les plus élevées. Dans une inflorescence donnée, les galles sont limitées aux six fleurs inférieures, avec environ 82 % des galles réparties entre la fleur la plus basse et celle située immédiatement au-dessus. Une telle répartition suppose une attaque du nématode en début de floraison. Le comportement observé pourrait dépendre de l'humidité disponible pour le nématode au moment de l'infestation. Les régulateurs de croissance de la plante pourraient également agir sur l'infestation et le nombre de galles.

Common fiddleneck, *Amsinckia intermedia* Fischer & Meyer (Boraginaceae), is a winter annual weed that is widespread in small grains, alfalfa, orchards, pastures and rangelands in California (Pantone, Brown & Womersley, 1985). Economic concern about its presence stems from the fact that the weed contains pyrrolizidine alkaloids which, if ingested, can cause hepatic unsufficiency, megalocytosis, and fibrosis in the livers of cattle, hogs, and horses (Fowler, 1968). Given the current regulations of herbicide use, there are few effective postemergent herbicides registered in California to control common fiddleneck in alfalfa (Pantone, Brown & Womersley, 1985). Thus, the development of biocontrol methods have become of paramount importance. In accordance with this, Nagamine and Maggenti (1980) suggested the potential importance of the nematode *Anguina amsinckiae* (Steiner & Scott, 1934) Thorne, 1961 as a biological weed control agent, but no research has been done to assess the practicality of this proposal.

Unlike some species of *Anguina* that are initially ectoparasitic but become endoparasitic on entering the developing inflorescence, *Anguina amsinckiae* is exclusively ectoparasitic and does not directly penetrate host tissues to initiate gall formation (Godfrey, 1940). Nematodes feeding on the immature inflorescence stimulates the primordial flower parts (petals, sepals, carpels and stamens) to become hyperplasic and surround the nematodes in a gall (Godfrey, 1940). Feeding continues within the gall, and reproduction occurs at the expense of the plant's own reproductive organs.

Our present research is concerned with assessing the potential of *Anguina amsinckiae* as a biocontrol agent for common fiddleneck. An important aspect of this is the observation that gall distribution within the inflorescence of the host plant is typically limited to the basal flowers (Steiner & Scott, 1934), suggesting that specific factors may operate to limit the extent of gall formation. We undertook the present work to investigate more fully this unusual distribution of nematode galls with a view to : *i)* gaining a deeper insight into host-parasitic relationships between *Anguina amsinckiae* and common fiddleneck and *ii)* enhancing the biocontrol potential of the nematode.

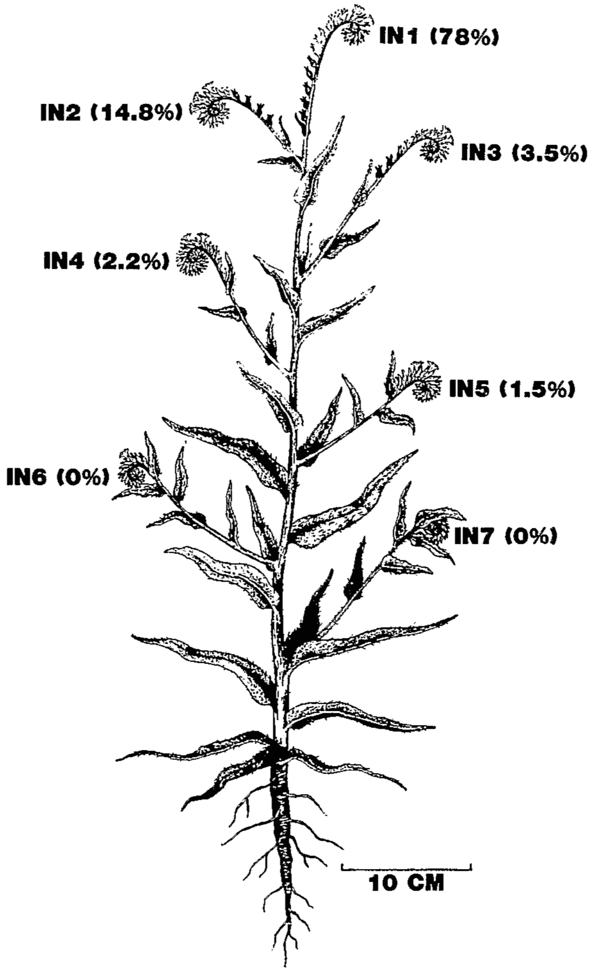

Fig. 1. *Amsinckia intermedia.* Inflorescence position coded by number (IN1, IN2, etc.). (Numbers in parentheses indicate the percentage of the total number of galls (n = 141) found within each inflorescence).

Materials and methods

On March 20, 1984, nematode-galled specimens of common fiddleneck were sampled at a site 24 km south of San Jose, California in the Santa Clara Valley (intersection of Monterey Road and Crowner Avenue). We believe that this is the site of nematosis that was investigated by Godfrey (1940) in 1936, 48 years prior to our studies. Using random number tables, the coordinates of a 6 × 6 meter plot were generated within the study site and all plants (n = 89) within the plot harvested. Each plant was examined for the presence of galls, and the size and position of each gall, the inflorescence in which it was situated, and the flower it replaced, recorded. Inflorescence and flower positions of each common fiddleneck individual were coded as follows : *a)* IN1-IN7, where IN1 represents the uppermost (most mature) inflorescence (Fig. 1); *b)* F1-F7, where F1 represents the lowermost (most mature) flower within a given inflorescence (Fig. 2 A). Common fiddleneck flowers are arranged in determinant inflorescences, referred to as helicoid cymes (Hitchcock *et al.*, 1969). Thus, the basally located flower is the oldest and blooms first (Fig. 2 A). A two-sample t-test that does not involve a pooled estimate of a common variance (Steel & Torrie, 1980) was used to compare the mean diameter of galls at different inflorescence and flower positions. In addition, a loglinear model (Fienberg, 1980) simultaneously tested the flower and inflorescence galling frequencies.

Results

Nematode-induced flower galls were found to be associated exclusively with the floral tissues, the degree of galling being affected by both the age of inflorescence and the age of flower within the inflorescence. Galls were restricted to the five most mature inflorescences in all plants studied. The uppermost inflorescence (IN1) contained 78 % of the galls found (Fig. 1). Gall incidence in the remaining infested inflorescences showed a marked decrease with IN2 containing 14.8 % and IN3, IN4, and IN5 collectively containing less than 8 % of the total galls (i.e. 3.5, 2.2 and 1.5 %, respectively). A similar pattern of gall distribution to that observed between inflorescences was also apparent within a given inflorescence. Only the six basally located flowers contained any galls, and approximately 82 % of the galls were located in either F1 or F2 (Fig. 2 A), these being the most mature flowers.

Galls formed on common fiddleneck by *Anguina amsinckiae* grow over a period of four to eight weeks to a maximum size of approximately 20 mm in diameter. An interesting trend can be seen in the size and position of the galls. Within IN1 the size of the galls decrease with increasing flower number (Fig. 3). Consequently, the mean gall diameter of IN1, F1 was larger than the

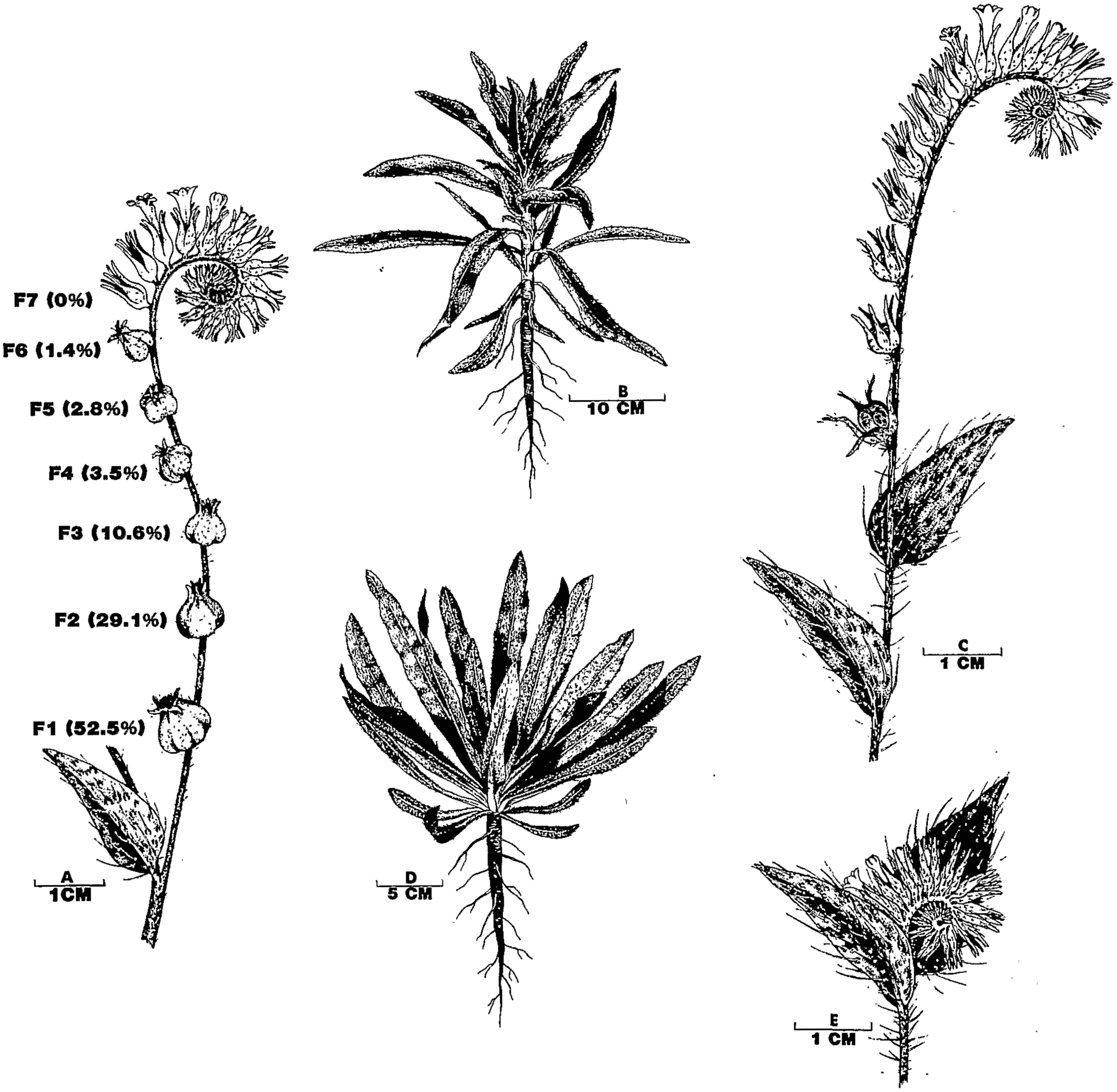

Fig. 2. *Amsinckia intermedia.* A : Flower position coded by number (F1, F2, etc.). F1-F6 as illustrated are replaced by galls. (Numbers in parentheses indicate the percentage of the total number of galls (n = 141) found in place of each flower.) B : Plant stature immediately prior to floral initiation; C : Form of a mature inflorescence (Note the seeds within the sepals of the lowermost flower : F1); D : Seedling or rosette stage; E : Form of a young inflorescence subtended by floral bracts.

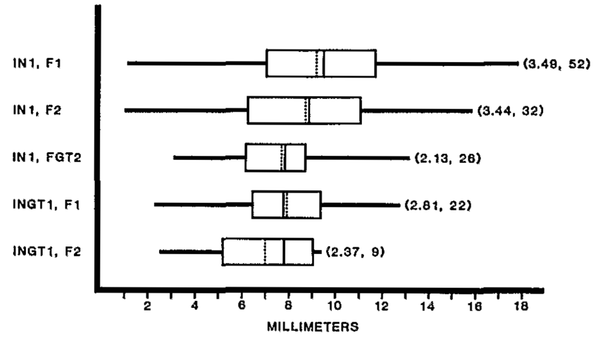

Fig. 3. Box and whiskers diagram of gall size measured by diameter. Dashed lines in the boxes indicate the sample means; solid lines in boxes indicate the medians. Fifty percent of the sample values are within the boxes; the left side of the boxes indicates the 25th percentile, and the right side of the boxes indicates the 75th percentile. The lines extending away from the edges of the box (whiskers) indicate to the left the minimum values and to the right the maximum values. Numbers in the parentheses are standard deviation and sample size (i.e. galling frequency), respectively. *(IN1 = Inflorescence 1; INGT1 = Inflorescence greater than 1; F1 = Flower 1; F2 = Flower 2; FGT2 = Flower greater than 2.)*

mean gall diameter of IN1, FGT2 (Inflorescence 1, Flower greater than 2) ($Z = 2.33$, $P < 0.05$). F1 is older than F2; F2 is older than F3, and so on. Therefore, the gall that replaces F1 is probably older than galls that replace F2 or F3. Older galls have had a chance to grow more and, therefore, are larger. The same analogy can be made for galls within different inflorescences but on the lowermost flower (F1). For example, the mean gall diameter of IN1, F1 was larger than that of INGT1, F1 (Inflorescence greater than 1, Flower 1) ($Z = 2.13$, $P < 0.05$).

Using a loglinear model which included the effects of inflorescence, flower, and inflorescence-flower interaction, we compared the frequency of nematode galls at different inflorescence and flower positions. The frequency data was grouped as indicated in Figure 3. Note that there were no galls within INGT2 at FGT2. The effect of inflorescence ($\chi^2 = 22.89$, d.f. = 1, $P < 0.0005$) was more important than flower ($\chi^2 = 8.44$, d.f. = 2, $P < 0.025$) in determining the gall frequency. No interactive effects between the inflorescence and flower were detected ($\chi^2 = 2.87$, d.f. = 2, P = n.s.). Four multiple comparisons were made : F1 and F2, F1 and FGT2, F2 and FGT2, and IN1 and INGT1. The F1 frequency was greater than F2 ($Z = 3.00$, $P < 0.05$) and FGT2 ($Z = 3.19$, $P < 0.05$). However, we were unable to conclude from the data that the F2 frequency was significantly greater than FGT2 ($Z = 0.66$, P = n.s.). Comparing the frequencies of galls within inflorescences, the IN1 frequency was greater than ING1 ($Z = 4.78$, $P < 0.05$).

Discussion

The success of *Anguina amsinckiae* in inducing gall formation seems to be dependent on its ability to locate susceptible flower primordia, since more mature flowers are probably immune to nematode infection (Godfrey, 1940). Statistical analyses of gall distribution demonstrate that the uppermost inflorescence and lowermost flower within an inflorescence are significantly more frequently galled by the nematode. This indicates that the observed gall distribution, and thus susceptible flower location, is not due to chance. Although it is probable that a number of factors influence the success of gall induction and its restriction to specific inflorescences and areas within them, our observations suggest that the most important factors are host growth patterns and moisture availability around the inflorescences.

A dramatic change in the form and stature of common fiddleneck occurs immediately after flowering is initiated. Prior to this, the weed is compact, the foliage dense, and the internodes are short (the rosette stage) (Fig. 2 D). It is presumed that the effects of environmental factors such as wind and sunlight would be reduced by virtue of this compact morphology, resulting in the maintenance of a relatively constant environment within the plant structure. In particular, the base of the rosette which is comprised of many whorls of tightly overlapping leaves that originate near the soil surface (Fig. 2 D) would appear to provide a high degree of protection from the effects of a fluctuating external environment compared to any other above-ground portion of the rosette. We have observed concentrations of nematodes on the moist surfaces of overlapping leaves in young seedlings close to the soil-plant interface (D. J. Pantone, unpubl.). However, this protection is subsequently reduced to a minimum at the initiation of and during flowering when the height of the plant increases two to threefold (Connor, 1965) with a concomitant increase in internode length and leaf spacing (Fig. 1). The effects of this, in terms of nematode displacement are probably twofold. Firstly, rapid stem elongation results in the total disruption of compact structure and the exposure of the aerial portion of the plant to a much harsher environment where wind and sunlight may reduce both free moisture on the plant's surface and the relative humidity of the surrounding air. Secondly, as stem elongation occurs, the sheathing and overlapping leaves present at the terminal growing tip of the stem (Fig. 2 B) may be instrumental in simultaneously providing shelter for infective nematodes and a means by which they are translocated to the earliest developing inflorescence (IN1). This is supported by the observations of Godfrey

(1940) who was only able to find groups of infective larvae lying between the young leaves of the terminal and lateral growing points during stem elongation.

With the above in mind, we hypothesize the following as being the probable mode of nematode infection, resulting in the observed distribution of galls. Initially, those nematodes that are able to locate the developing apex of the plant at the onset of stem elongation will tend to be within the protected environment provided by the young leaves (Godfrey, 1940). Thus, even though stem elongation disrupts compact structure (Fig. 2 B), the shelter provided by the leaves at the apex of the stem ensures that high relative humidities and surface water are maintained within the apically developing inflorescence (IN1). This in turn will allow a favorable environment for infective nematodes, and will favor gall formation on the earliest developing flowers. Subsequently, as IN2 begins to form, the continued change in plant morphology will permit further removal of surface moisture and restrict the movement of nematodes either from IN1 or from the base of the plant to IN2. With continued growth, the extent of surface water loss by evaporation will be such that at the time of IN6 and IN7 development (Fig. 1), nematode movement will be totally curtailed, resulting in the noninfection of otherwise susceptible floral meristems.

A similar scenario may also explain the preferential galling of the lowermost flowers (F1-F6) within a given inflorescence. The F1 forms before the cymose inflorescence elongates (Fig. 2 E). Analogous to the conditions that occur when IN1 is forming, the tightly curled inflorescence and floral bracts that occur when F1 first forms provides a microenvironment that is sheltered from the effects of desiccation. Later as the inflorescence elongates (Fig. 2 C), the floral meristems will be subjected to increased exposure to the external environment, resulting in a reduction in relative humidity near the flowers, curtailing nematode movement.

Godfrey (1940) reported that *Anguina amsinckiae* stimulates the floral meristems to differentiate into galls by ectoparasitic feeding. Apparently, only very young flowers whose tissues have not differentiated into more mature floral organs are susceptible. If *A. amsinckiae* is to cause the flower to form a gall, the nematode must feed on meristematic floral tissues. Consequently, the period when the flower is susceptible is probably quite short. If the maturation of the floral meristems could be slowed, the period of susceptibility to nematosis may be lengthened, and the incidence of galling increased. Plant growth inhibitors may provide a means of artificially slowing down floral maturation and thereby enhance the degree of galling.

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