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Developmental variation in a species of *Isoglossa* (Acanthaceae: Ruellioideae) over a season

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ABSTRACT

In his appraisal of *Isoglossa* Oerst., Clarke (1901) placed major emphasis on the sizes of leaves and inflorescences in the key to species, but efforts to sort herbarium material have shown a great deal of variation in leaf size and inflorescence length that is very difficult to interpret. Specimens of a species of *Isoglossa* were collected from wild subpopulations from the Hartebeespoort Dam and Hartebeeshoek areas, at intervals over a season. Leaves and inflorescences were measured, and frequencies of hair types on bracts and calyces were recorded photographically. Results indicate that leaf size within a species increases over the vegetative phase of the plant and that inflorescence size within a species increases over the reproductive phase of the plant. Therefore, caution should be applied when these characters are used for species identification in *Isoglossa*. Bract and calyx indumenta also change over a season, thus making it necessary to use wisdom when applying this character taxonomically.

INTRODUCTION

Isoglossa Oerst. is a member of the pantropical family Acanthaceae, with about 50 species in the Old World tropics (Balkwill & Welman 2000). Most genera within the Acanthaceae include perennial herbs, subshrubs and shrubs but some members are monocarpic. Daniel (2006) has undertaken a review and provided references that record monocarpy in *Acanthopale* C.B.Clarke, *Aechmanthera* Nees, *Stenosiphonium* Nees, *Strobilanthes* Blume and *Mimulopsis* Schweinf. within the Acanthaceae. In the genus *Isoglossa*, monocarpy has been recorded for *I. woodii* C.B.Clarke (Clarke 1901; Van Steenis 1978), *I. substrobolina* C.B.Clarke and *I. oerstediana* Lindau (Tweedie 1976). Therefore, recording changes in leaf and inflorescence size of a wild subpopulation of a monocarpic species of *Isoglossa* over a season will provide an understanding of the nature of morphological variation and characters that are suitable for use in a re-appraisal of species circumscriptions in *Isoglossa*.

In general, perennial plants are morphologically relatively constant over a flowering season, so that herbarium specimens of perennials show consistency, even if collected at different periods over a season. In contrast, however, annuals, short-lived perennials and monocarpic species often show great morphological variation over a season because of the high level of translocation of nutrients from vegetative structures into the reproductive organs. Herbarium specimens of the same species may thus show considerable morphological differences depending on whether they were collected at the beginning or near the end of the flowering/fruiting season.

Indumenta of bracts and calyces are regarded as being taxonomically important within the Acanthaceae and are used in Clarke's (1901) key to species in the genus *Isoglossa*. In some cases, the difference in indumentum may be genetically determined [e.g. *Isoglossa vulcanicola* Mildbr. has a variety that is characterized on the basis of being eglandular (Mildbraed 1943)], whereas in others the difference may be developmental or environmental. Observation of these characters within a subpopulation will clarify how they vary and whether or not the variation is genetically fixed and thus whether it is taxonomically useful.

Many more herbarium specimens of *Isoglossa* exist now than were available to Clarke and it is difficult to group them into Clarke's (1901) taxa because it seems that he did not take the probable morphological implications of monocarpy into account when delimiting species. So a vital step in establishing useful circumscriptions and ranges of measurements for species of *Isoglossa* is to determine how the developmental and translocational processes associated with monocarpy would influence the morphology expressed by a single genome over the course of a flowering season. This paper provides insight into meaningful ranges of measurements of structures in monocarpic taxa and thus for the evaluation of Clarke's species circumscriptions.

An annual or monocarpic plant will show several stages in its development with various nutrient balances and fluxes resulting in various morphological consequences. In the initial stage, nutrients will be translocated from the cotyledons to form the radicle and plumule. Very soon, the first leaves will begin to photosynthesize and in this vegetative phase photosynthate will be used in the elaboration of the root system to absorb water and minerals and the shoot system to provide a framework as well as leaves to photosynthesize. Over the course of this phase, the leaf size is likely to increase. When the plant enters the reproductive phase, new framework is produced to support the inflorescences and leaves are often associated with these new nodes. In addition, bracts, bracteoles and calyces are produced, which will usually complement the photosynthetic function of the leaves and will compete with the new leaves for photosynthate during the development of both reproductive and vegetative organs. Consequently, the leaves

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formed at this stage are smaller than those formed at the peak of the vegetative phase. Once anthesis begins, most of the structures that are produced (i.e. corollas, stamens, pollen, ovules) are not photosynthetic and are thus sinks for, rather than sources of, photosynthate. At this stage, the nutrients from the older leaves are often translocated into these sinks, especially once fruits and seeds are being produced. The older leaves (including the larger ones) will be abscised once they no longer provide nutrients and the plants will be left with the smaller leaves that were formed at the onset of the reproductive phase.

Many theories exist to explain leaf growth and although some may be inter-related, the debate remains unresolved (Inzé 2003). One such theory is the organismal one, which implies that there is a set number of cells per leaf and that leaf growth is accounted for by cell expansion (Beemster *et al.* 2003; Inzé 2003); i.e. by an increase of cell water content through vacuolation (Beemster *et al.* 2003). The behaviour of leaf cells is orchestrated at organ level and leaf size at whole plant level (Tsukaya 2003). In contrast, the cell theory proposes that leaf growth is due to an increase in cell number, with the size of the cells remaining constant (Tsukaya 2003; Cookson *et al.* 2006). Large mature leaves formed in the manner implied by the organismal theory would require less photosynthate to produce than leaves of similar size formed in accordance with the cell theory where there would be a considerably greater volume of cell wall. However, the organismal theory would not explain why smaller leaves are produced at the onset of the reproductive phase.

Since 'the number of chloroplasts per cell remains approximately constant' (Taitz *et al.* 1991), an increase in leaf size due to an increase in cell number will proportionately increase the number of chloroplasts and thus the amount of photosynthate produced per leaf. In contrast, an increase in leaf size attributed to an increase in cell size would reflect no increase in chloroplast number and correspondingly little or no increase in photosynthate production. Thus, leaf cell counts done at crucial developmental stages over a season may reflect on the interaction of photosynthate production and translocation as well as which theory explains leaf growth in this monocarpic species.

The species circumscription and nomenclature within southern African *Isoglossa* has not as yet been finalized. Most specimens of the species under study in this paper have been identified as *Isoglossa stipitata* C.B.Clarke. Clarke (1901) cites one synonym, two misapplied names and two specimens under his *I. stipitata*. His name should be typified on the type of the synonym, *Rhytiglossa glandulosa* Hochst. Hochstetter's (1845) type is *Krauss 302*, which would have been in Berlin and thus destroyed during World War II. Clarke (1901) assumed that the *Krauss 502* specimen of *Isoglossa* at Kew was Hochstetter's type, which had been mistakenly distributed with the number 502 instead of 302. We have obtained a *Krauss 302* specimen from Missouri (originally from The Bernhardi Herbarium) which is different from the *Krauss 502* that was annotated by Clarke, at least in having well-spaced nodes in the inflorescence, a feature captured in Hochstetter's description. Thus, Hochstetter's name, of which Clarke's *I. stipitata* is strictly a later synonym, should be applied to an entity which is either closely allied to or conspecific with the well-known monocarpic understorey shrub of the KwaZulu-Natal coast, *I. woodii* C.B.Clarke. Clarke's (1901) description of *I. stipitata* matches the *Krauss 502* specimen which he cites and we consider this to most likely be conspecific with *I. woodii*.

Flanagan 2322, the other specimen that Clarke cites, agrees with the species we have worked on, but differs from Hochstetter's and Clarke's descriptions of *Rhytiglossa glandulosa* and *Isoglossa stipitata* and *Krauss 302* and *502*, by having lanceolate rather than obovate, acuminate bracts. Our species also does not agree with the types of the two misapplied names that Clarke cites and we thus conclude that it has yet to be described and refer to it as *Isoglossa* sp. 1.

Clarke's specimens of *Isoglossa stipitata* occur only at the coast, whereas our specimens are found inland and at the coast. *Isoglossa* sp. 1 is the most widely distributed of the southern African members of *Isoglossa* (Figure 1), and occurs along the coast from the Eastern Cape Province to eastern Kwazulu-Natal, as well as inland from Barberton in Mpumalanga, through Gauteng to the North-West Province (Sebola 1994).

MATERIALS AND METHODS

Isoglossa subpopulations from two localities in the North-West Province (Sandspruit Farm, 2527DB, near Hartebeespoort Dam and Sugar Bush Mountain L49, 2527DD, near Hartebeeshoek) (Figure 1) were sampled at approximately three-week intervals, from January 2005 to August 2005. Due to the monocarpic nature of the plant, which dies after bearing fruit, no samples were taken in September or October 2005 (characteristically dry months). Sampling recommenced two weeks after the first rains in November 2005 and continued until March 2006 but these were specimens from a subsequent generation of plants.

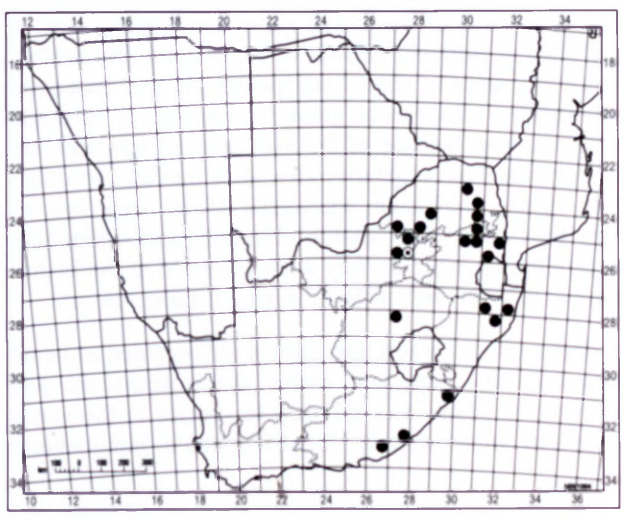

FIGURE 1.—Map showing distribution of *Isoglossa* sp. 1, ●; as well as sampling positions at Hartebeeshoek, Sugar Bush Mountain and Hartebeespoort Dam, Sandspruit Farm, North-West Province, ⊙.

FIGURE 2.—Specimens of branches of *Isoglossa* sp. 1 showing stages of development over a season: leaves subtending lower branches have dropped off in Dec. as in B, this process continues including leaves beginning to drop off axillary branches in May as in H, all leaves have dropped off by Aug. as in I. A, 19 Dec. 2005; B, 9 Jan. 2006; C, 16 Feb. 2006; D; 1 Mar. 2006; E, 6 Mar. 2005; F, 20 Mar. 2005; G, 8 Apr. 2005; H, 2 May 2005; I, 20 Aug. 2005. Scale bar: 55 mm.

It was extremely difficult to identify *Isoglossa* prior to flowering and field trips in November yielded no specimens of *Isoglossa*. The four neighbourhoods of *Isoglossa* at Hartebeeshoek did not re-appear after the first rains in November 2005. As a result, it was initially believed that *Isoglossa* may be locally extinct at this locality. However, an extensive search of the area in late March 2006 revealed a single plant growing close to the skeletons of what must have been a previously undiscovered fifth neighbourhood. This plant was growing in denser shade on the opposite, eastern side of the dry stream bed. This may account for its survival. It is possible that the seedlings of the first four neighbourhoods died after germinating, as there was a dry spell between the first rains

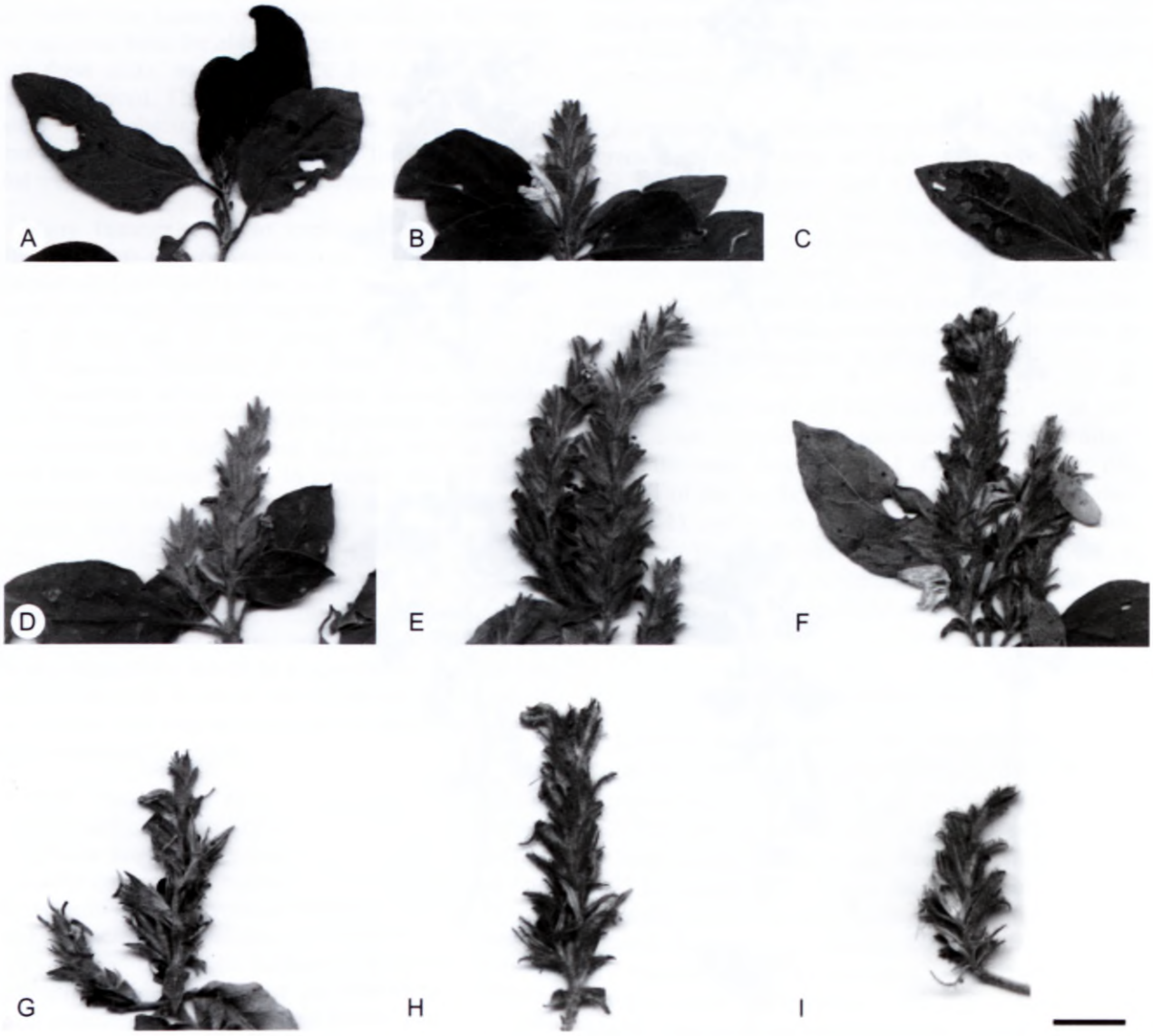

FIGURE 3.—Development of inflorescences over a season: A, 9 Jan.; B, 1 Feb.; C, 1 Mar.; D, 20 Mar.; E, 8 Apr.; F, 2 May; G, 20 May; H, 12 Jun.; I, 20 Aug. B, C, H, terminal inflorescences; D–G, inflorescences both terminal and lateral; I, lateral inflorescence. Inflorescence development begins in Jan. with length peaking in June. All to same scale. Scale bar: 10 mm.

and subsequent more frequent rains. This may not have affected the Hartebeespoort subpopulation, which experienced slightly later, frequent rain. As a result, only those specimens collected from the Hartebeespoort locality were used in the analyses. The loss of the four neighbourhoods from the second locality caused unintended limitations to the methodology, because it would have been preferable to have been able to study more than one subpopulation.

Where possible, two specimens per sampling period were cut from plants with the most developed branches within the subpopulation. Specimens were pressed in a plant press, dried at 37°C for a three-day period, then sterilized in a deep freeze for two days, before being brought into the herbarium where they were photographed and measured.

Specimens were scanned with an Epson Expression 1640XL scanner, using the same frame size and resolution each time to ensure consistent magnification. The length of the six largest leaves and the longest lateral inflorescences were measured on each of the pairs of specimens collected over the sampling period. During the months of June to August, the number of leaves measured per specimen decreased, as the leaves are shed during these dry winter months and nutrients are translocated to the reproductive organs. Terminal inflorescence lengths were measured—the number of measurements varied depending on the number of terminal inflorescences on each specimen. Using the programme Statistica, the mean, minimum and maximum lengths together with the standard deviation were calculated for each sampling period (Figures 4–6). Leaf variation over a season was determined by comparing the mean of the shortest leaves produced to the mean of the largest leaves produced within a subpopulation in a season. Both terminal and lateral inflorescence variations over a season were calculated in the same way. Whereas the means in Figure 4 are based on up to six leaves from

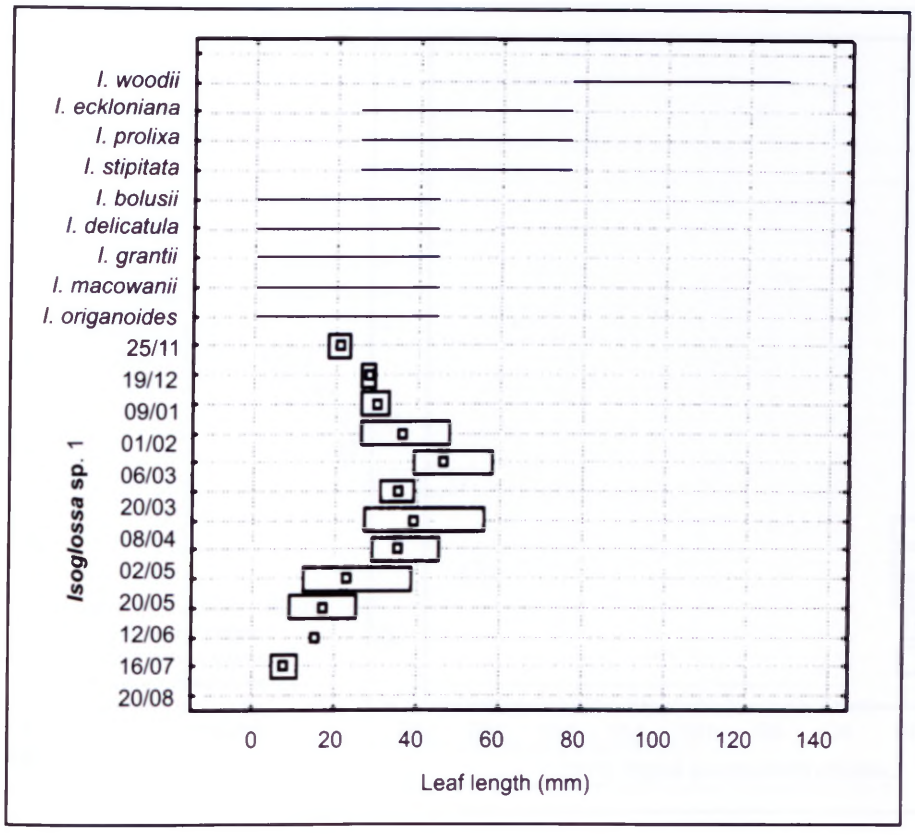

FIGURE 4.—Leaf length (mm) of *Isoglossa* sp. 1 measured over a season compared to leaf length ranges of Clarke's species mentioned in his key and descriptions. Single lines show ranges cited by Clarke (1901). Boxes are leaf length measurements in a natural subpopulation of *I.* sp.1 on dates indicated (n = 6). Leaf length of *I.* sp. 1 reaches a maximum in March. Leaf lengths of *I.* sp. 1 straddle boundaries of leaf lengths of 2 of 3 groups of Clarke's species. □, mean; ⊓, min.-max.; ⊥, SD.

two specimens, Figure 2 illustrates only one of the two specimens from any collecting period. A similar situation exists between Figures 5 and 6 and Figure 3.

Photographs to show the indumentum of the bracts and calyces at various stages of development (Figure 7) were taken using a Nikon SMZ 1500 dissecting Microscope and Nikon Digital Camera DXM 1200 attachment. Entire plants (Figure 8) were photographed using a Nikon E 990 digital camera to show morphological changes over a season as well as various habits.

Cell counts were calculated from four mature leaves sampled on the 9 January 2006 (representing leaves that were first produced), 6 March 2005 (representing the largest leaves produced) and 2 May 2005 (representing the smaller leaves that were produced later during inflorescence development) to determine whether the increase in leaf size over a season was attributable to an increase in leaf cell numbers (indicating greater producution of photosynthate). Similarly, a decrease in total cell numbers in the smaller leaves that are produced later in the season, would indicate that the photosynthate initially produced by the larger leaves is utilized mainly for inflorescence development. Since only two specimens were collected per sampling date, only four leaves were used for the cell counts, corresponding to the two most mature leaves on each specimen collected. The specimens from 9 January 2006 were used for the leaf cell counts representing leaves that are first produced, as although specimens were collected in December 2005, leaf clearing did not produce consistently visible cells.

A modified leaf-clearing technique (O'Brien & von Teichman 1974) was devised to make cell counts possible. The instructions for normal specimens (O'Brien & von Teichman 1974) were followed, with the exception that the leaves were placed in a 5 % potassium hydroxide (KOH) solution at room temperature following the 80 % ethanol autoclave procedure. The leaves used were from the dried samples, which may account for why initial experiments revealed that the leaves disintegrated or became unmanageable when autoclaved with 1 % KOH. Additionally, varying autoclave times of both the 80 % ethanol and 1 % KOH did not produce the required clearing results. Time durations for leaf submergence in the 5 % KOH varied, according to leaf size (leaf area 391 mm^2—36 hours, 627 mm^2—49 hours, and 140 mm^2—30 hours). When a leaf looked clear it was removed from the KOH solution, subjected to several distilled water washes, mounted on a glass slide and observed under a compound microscope. If cells were not visible, the leaf was replaced in the KOH solution and the same procedure was repeated at regular intervals. Once cells were visible, the leaf was stained with basic fuschin (O'Brien & von Teichman 1974).

Leaves and a ruler (for scale) were scanned with an Epson Expression 1640XL scanner, and the programme Simple PCI was used to calculate leaf areas. Three constant sites per abaxial leaf surface were viewed under an Olympus BH-2 compound microscope, linked to a Nikon Digital Camera DXM 1200 and computer viewing screen. A stage micrometer was used to determine the dimensions of the image and hence to calculate the area (0.04 mm^2) projected on the computer screen. The same microscope magnification (× 20) and resolution settings (Quick 3, 1280 × 1024) were used throughout, for each of the sites and for each of the leaves. Each site was positioned equidistant from the midrib and leaf edge, with one site positioned centrally on one half of the leaf. Each of the other sites was positioned on the opposite side of the midrib, halfway between the mid-

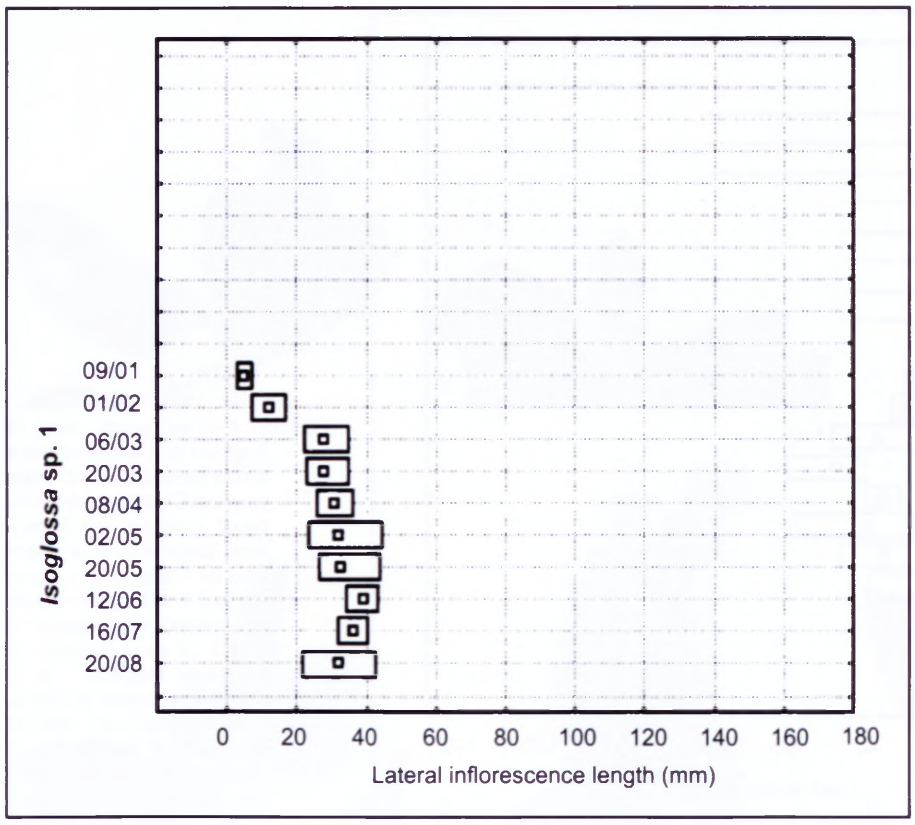

FIGURE 5.—Lateral inflorescence length (mm) of *Isoglossa* sp. 1 measured over a season. Boxes are measurements made in a natural subpopulation on dates indicated (n = 6). Inflorescence development begins in Jan. and reaches its peak in June. □, mean; ⊓⊔, min.-max.; ⊥, SD.

point and the base or apex of the leaf. This sampling procedure standardizes cell size variation in different areas of the leaf (Horvàth *et al.* 2006). The programme Act 1 enabled photography and subsequent cell counting per site, with the counts based on Gundersen's 'unbiased two-dimensional sampling rule' (Kubíniová 1994; Gundersen 1997). Both epidermal and stomatal cells were included and since the variation in the cell numbers per site in each leaf was very slight, the numbers were averaged. The following equation rendered the total number of cells per leaf:

$$\frac{\text{leaf area}}{\text{site area}} \times \text{mean no. cells per three sites}$$

Since more than one leaf was cleared per sampling date, the mean and standard deviation for the total number of cells per leaf was calculated using the programme Quattro Pro (Table 1). Cell areas were calculated by dividing each of the three site areas (0.04 mm²) per leaf by the number of cells per site and the mean and standard deviation for the total cell area per four leaves sampled was calculated (Table 1). A t-test (using two tails and two sample variance) between the means of the cell areas for leaves sampled on the 9 January and the 6 March, and those on the 6 March and on the 2 May, was carried out using the programme Epistat.

RESULTS AND DISCUSSION

Isoglossa sp. 1 developed a tall (1–2 m high), scandent habit in the shade (Figure 8B). It had a softer stem and used woody branches from surrounding trees for support. This enabled it to obtain more light. Plants in the same subpopulations growing in brighter light were not as tall and had more sturdy stems (Figure 8C).

Four phenological trends were identified over the study period. In December the plants increased in size and branching (Figure 2), followed by the development of inflorescences in January (Figure 3). The first trend involved an increase in inflorescence length, which reached a maximum in June, together with the development of fruit (Figures 3A–H). Thereafter, there was a general waning of the subpopulations until August where senescence was prevalent. The bare brown branches were still in evidence the following season (Figure 8D).

The second trend was that leaves began to fall off the main stem as early as February (Figure 2C) and the plant continued to lose its leaves until May, when only the leaves below the spikes were evident. By August (Figure 2I), hardly any leaves were present. A caterpillar infestation was apparent in the leaves in February and was noted in both years at this time.

The third trend involved changes in bract indumentum. Observation of the inflorescences showed that the bracts of the young spikes were white-ciliate (Figure 7A). As the spikes increased in length and the bracts diverged, a combination of both eglandular and glandular trichomes became evident (Figure 7B, C). By April (Figure 7D) and the onset of fruit production, the gland tips were brown and stickier to the touch. By June (Figure 7F), they were extremely sticky. As the seed was dispersed, the bracts, calyces and trichomes senesced.

The fourth trend was that the leaves that were first produced (i.e. 9 January\2006) had a lower mean cell count per site area than those produced later, i.e. the cell area was slightly larger (Table 1). A t-test between the mean cell areas of the mature leaves that were first produced (9 January) and the largest leaves produced (6 March) showed a significant difference, with t =

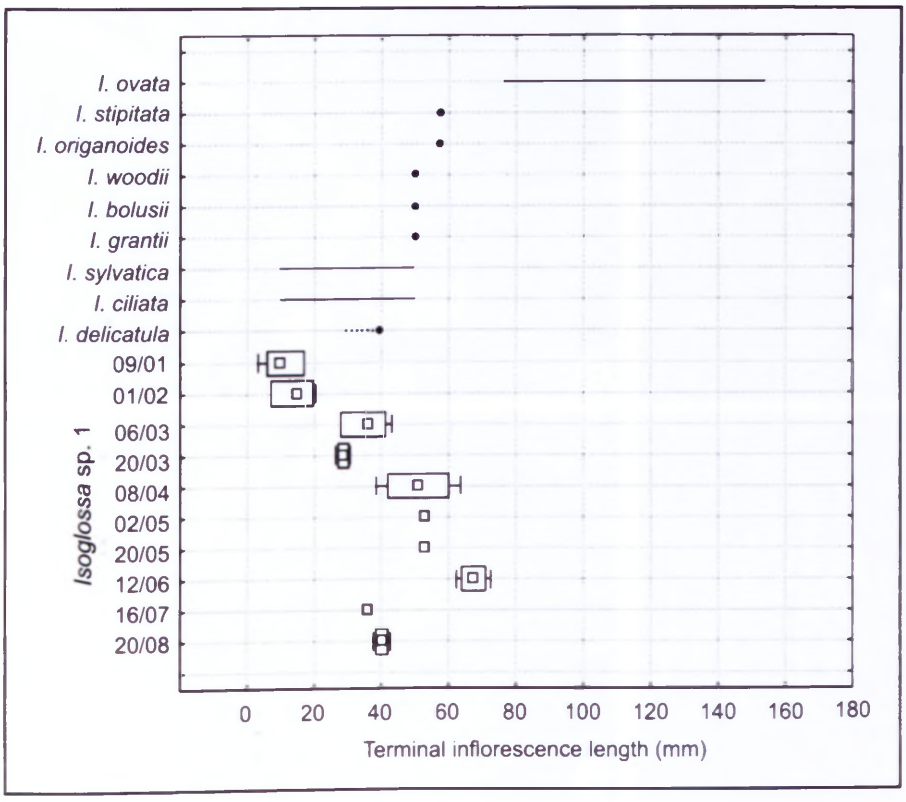

FIGURE 6.—Terminal inflorescence length (mm) of *Isoglossa* sp. 1 measured over a season, compared to inflorescence length ranges of Clarke's (1901) species. Single and dotted lines show ranges cited by Clarke: dotted line indicates mostly shorter than length cited; points are single lengths mentioned in Clarke's species descriptions. Box and whiskers are measurements made in a natural subpopulation of *I.* sp. 1 on dates indicated. Inflorescence lengths of *I.* sp. 1 straddle boundaries of inflorescence lengths of 3 out of 4 groups of Clarke's species. □, mean; ⊓, min.-max.; ⊥, SD.

8.903395 and $P < 10^{-6}$. However, these cells were not as definable as those in the later samples, and thus counts may be underestimated. Results of a t-test between the means of the cell areas for the largest leaves produced (6 March) and the smaller leaves produced later (2 May), with $t = 1.172771$ and $P = 0.2534304$, show no significant difference between these samples, which is consistent with the cell theory, where the cell size remains fairly constant.

Morphological development of leaves

The mean leaf length at the beginning of the season was 20.5 mm, peaking at 46 mm in March and was 6.5 mm at the end of the season, which gave a 39.50 mm variation (Figure 4).

The girth of the stem and the length of internodes appear to remain constant over the season, but leaf size varies considerably. Mean leaf length increases and

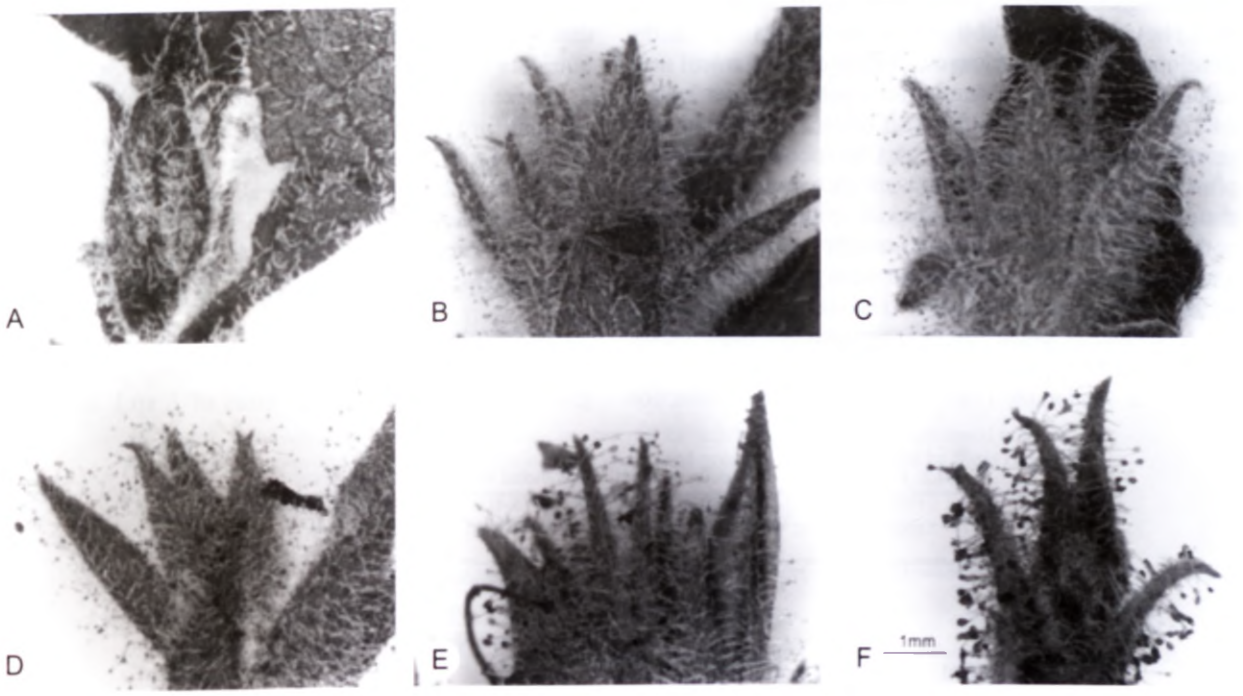

FIGURE 7.—Indumentum of bracts and calyx lobes over a season: A, Jan. 2006; B, Feb. 2006; C, Mar. 2006; D, Apr. 2005; E, May 2005; F, Jun. 2005. Closed bracts are white ciliate in Jan. Bracts diverge in Feb, revealing mix of translucent glandular and eglandular trichomes on bracts and calyces. Glandular trichomes become increasingly viscid from April until June. All to same scale. Scale bar: 1 mm.

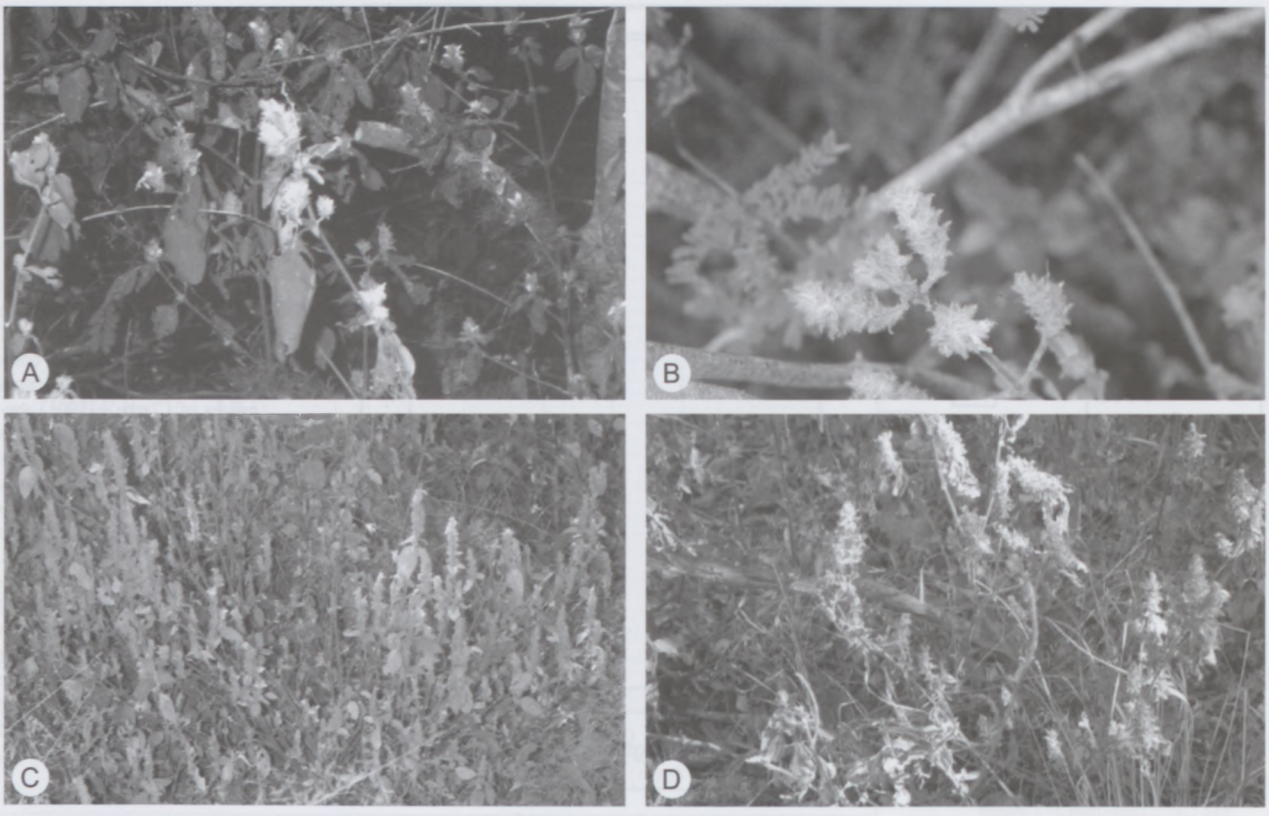

FIGURE 8.—Morphological changes over a season as well as various habits of *Isoglossa* sp. 1. A, April, 1–2 m tall, scandent habit in shade; B, April, extremely viscid inflorescence; C, May, growing in brighter light, not as tall, sturdier stem; D, Aug. inflorescence on dry, bare branches.

then decreases with time (Figure 4). The increase in the mean leaf length (Figure 2) is consistent with the suggestion that early in the season, photosynthate (initially produced by the cotyledons) is used for leaf production on the major branches. The leaf area for leaves that are first produced (9 December) is 359.75 ± 9845 mm² and corresponding cell counts of 112355 ± 11225 (Table 1). Increasing numbers of leaves led to an increase in available photosynthate, and therefore more nutrients for the development of larger leaves, evidenced by the leaf area being 743.75 ± 195.22 mm² with a cell count per leaf of 343323 ± 102779 on 6 March (Table 1). This increase in cell number per leaf indicates an increase in chloroplast numbers per leaf and thus an increase in photosynthate production. Although abaxial epidermal cells were counted, it is assumed that the number of palisade cells and thus the numbers of chloroplasts would be proportional to the number of epidermal cells. Later, during the reproductive phase, there is competition between the leaves and the reproductive structures for photosynthate (Meyer & Anderson 1949) resulting in the production of smaller mature leaves, with the leaf area falling to 340.50 ± 73.79 mm² and cell count to 264925 ± 125194 (2 May), (Table 1). Variance in leaf size for 6 March is accounted for by there being only two specimens per sampling period, which subsequently explains the relatively high standard deviation for the mean total leaf cell numbers calculated (Table 1). The same is true for leaves sampled on 2 May. In addition, the larger leaves that were formed in the vegetative phase are divested of nutrients and thus they senesce and are dropped (Figure 2). These changes in leaf size and their deciduous nature account for the apparent decrease in the mean leaf length (Figure 4). The cell numbers counted in mature leaves of different sizes support the cell theory (Tsukaya 2003) of leaf enlargement and of the hypothesis of competition for photosynthate resulting in smaller mature leaves being produced during the reproductive phase.

Measurements gleaned from Clarke's (1901) descriptions and key for *Isoglossa* (see below) produce three groups of species, with leaf sizes of *I.* sp.1 in the field

TABLE 1.—Leaf area, cell counts and cell areas based on three measurements (from middle, base and apex) on each of four leaves (i.e. n = 12). Leaf cell counts increase during vegetative phase, reaching maximum in March

	Leaves produced		
	First–9 Jan.	Largest–6 March	Smaller–2 May
	Mean and standard deviation		
Cell count per 0.04 mm²	11 ± 2	18 ± 4.8	18 ± 4
Cell area mm²	0.0036 ± 0.0005	0.0022 ± 0.0002	0.0024 ± 0.0005
Leaf area mm²	359.75 ± 89.45	743.75 ± 195.22	340.50 ± 73.79
Total leaf cell count	112355 ± 11225	343323 ± 102779	264925 ± 125194

straddling the boundary between two of the groups (Table 2, Figure 4). The field data can be more closely associated with the group with smaller leaves than with the group with leaves of intermediate size. In fact, Clarke's data for *I. stipitata* reflect a greater leaf length than the largest leaf size recorded in the field. Although it is likely that subpopulations of the species growing in more mesic climates would have larger leaves, no direct comparisons can be made as our *I.* sp. 1 is not equivalent to Clarke's *I. stipitata*. Clarke differentiates *Isoglossa woodii* from other species in the key on the basis of its extremely long leaves (Figure 4) and in addition he quotes the maximum leaf lengths for *I. hypoestiflora*, *I. sylvatica*, *I. ovata* and *I. cooperi* in the descriptions. The considerable variation observed in leaf length of *Isoglossa* over a season (Figure 4) suggests that leaf length alone should not be used for species determination within *Isoglossa* and other monocarpic species. Further, a comparison of leaf length measurements taken from Clarke's key with those taken from individual species descriptions (Figure 4) shows slight anomalies, in that Clarke states 'leaves up to $1^3/_4$ inches' in his species description for *Isoglossa delicatula*, whereas, in the key (see below) it is grouped with species with leaves 'attaining $1–1^1/_2$ inches' in length.

Clarke's (1901) key to species of *Isoglossa*
(Copyright protection has expired—in the Public Domain, 1957)

Subgenus 1. *Eu-Isoglossa*. Corolla $^1/_3–^2/_3$ in. long; tube cylindric:
- Calyx with hairs none gland-headed:
  - Inflorescences $^1/_2$–2 in. long:
    - Bracteoles not white margined ..... (1) *ciliata*
    - Bracteoles white-margined ..... (2) *sylvatica*
  - Inflorescences 3–6 in. long..... (3) *ovata*
- Calyx with numerous gland-headed hairs:
  - Leaves large, some up to 5 by 2–3 in. ..... (4) *woodii*
  - Leaves medium-sized, some up to 3 by $1–1^1/_2$ in.:
    - Flowers very loosely paniculate, nearly all solitary.....(5) *prolixa*
    - Flowers approximated:
      - Bracts linear-lanceolate .....(6) *eckloniana*
      - Bracts obovate, obtuse with a short acumination ... (7) *stipitata*
  - Leaves attaining $1–1^1/_2$ in. in length, mostly shorter:
    - Spikes slightly interrupted, manifestly viscid-hairy:
      - Bracts $^1/_2–^2/_3$ in. long, broadly lanceolate .....(8) *origanoides*
      - Bracts $^1/_4–^1/_3$ in. long, spathulate-obovate ..... (9) *grantii*
      - Bracts $^1/_8–^1/_6$ in. long, oblong .....(10) *bolusii*
    - Spikes interrupted, loose, sparingly hairy (except the calyx):
      - Corolla $^3/_4$ in. long, rather broad.....(11) *macowanii*
      - Corolla $^1/_3–^1/_2$ in. long, slender..... (12) *delicatula*

Subgenus 2. *Ramusia*. Corolla $1^1/_2$ in. long; tube slender:
- Bracteoles and calyx thinly hispid with white non-glandular hairs ..... (13) *hypoestiflora*
- Bracteoles and calyx viscid with many gland-headed hairs ..(14) *cooperi*

Morphological development of inflorescence

The mean lateral inflorescence length at the beginning of the season was 6.00 mm and increased to 39.8 mm showing a 33.8 mm variation over the season (Figure 5). Similarly, the mean terminal inflorescence length was initially 7.5 mm in January and reached a maximum mean length of 68 mm, showing a 60.5 mm increase over the season (Figure 6). Terminal inflorescences are generally longer than the lateral inflorescences (Figures 5, 6) with the anomalies probably accounted for by the low number of terminal inflorescences found on some specimens. It should be noted that *Isoglossa ovata* has predominantly terminal inflorescences which is characteristic of that species. Both terminal and lateral inflorescences show a decrease in length in July and August (Figures 5, 6). This may be attributed to variation found within a subpopulation, i.e. plants that have developed later in a season when conditions are less favourable produce smaller infloresences, or to these specimens possibly having grown in sunnier conditions.

In monocarpic plants, leaf loss is associated with the nutrients being translocated for reproduction (Wareing & Phillips 1970). Our studies show that leaf loss begins in February and continues to May (Figure 2). Inflorescence development begins in January (Figure 5), with inflorescence length increasing over a season and reaching a peak in June. Thus, relatively late inflorescence development (Figures 2, 3) suggests that photosynthate is first used for leaf production, then production of larger leaves (supported by an 227144 increase in leaf cell numbers) (Table 1) and lastly as the leaves senesce, the photosynthate is translocated for inflorescence development. A comparison of inflorescence length over a season (Figure 6) with Clarke's inflorescence ranges shows that Clarke's species form four groups (Table 2). *Isoglossa ovata* is characterized by an extremely long inflorescence and is entirely separate from the other groups and from the field data. The second group of species includes *I. stipitata* and *I. origanoides*. *I. bolusii*, *I. grantii*, *I. woodii*, *I. sylvatica* and *I. ciliata* form the third of Clarke's groups. This group, together with the fourth group, comprising *I. delicatula*, fall within the boundaries for the field data of *I.* sp. 1. Although it should be noted that Clarke (1901) only uses inflorescence length to diagnose *I. ovata* in the key, he does quote inflorescence lengths for the previously mentioned species in the descriptions. The variability in inflorescence length suggests that it should be used with caution as a taxonomic character for species delimitation within *Isoglossa* and in future studies of other monocarpic plants.

TABLE 2.—Conversion of imperial to metric measurements, for inflorescence and leaf lengths used in Clarke's (1901) key to and descriptions of species of *Isoglossa* and groups of species defined by these characters

Imperial	Metric	Clarke's (1901) *Isoglossa* spp.
Inflorescence		
$^1/_2$–2 inches	12.7–50.8 mm	*I. ciliata*, *I. sylvatica*, *I. bolusii*, *I. grantii*, *I. woodii*
$^1/_2–2^1/_2$ inches	12.7–63.5 mm	*I. origanoides*, *I. stipitata*
3–6 inches	76.2–152.4 mm	*I. ovata*
Leaves		
Up to 5 inches	Up to 127 mm	*I. woodii*
Up to 3 inches	Up to 76.2 mm	*I. prolixa*, *I. eckloniana*, *I. stipitata*
Up to $1–1^1/_2$ inches	Up to 25.4–38.1 mm	*I. origanoides*, *I. grantii*, *I. bolusii*, *I. macowanii*, *I. delicatula*

Micromorphological development of indumentum on bracts and calyces

A comparison of bracts over a season (Figures 7A–F) reveals that the proportion of glandular trichomes increases on individual bracts over a season. Further, the increase in degree of viscidity of the glandular trichomes may exist as a means of protection for the fruit against predation. Although the developmental variation in proportion of glandular hairs may question the taxonomic usefulness of the indumentum in Clarke's (1901) key to species of *Isoglossa*, he does use the indumentum in conjunction with other characters. Our findings suggest that it is important to compare the various kinds of trichomes in species at comparable developmental stages, especially when using the indumentum as a taxonomic character in monocarpic plants.

CONCLUSION

Results showed that the mature larger leaves had more cells than the mature smaller leaves which supports the cell theory (Tsukaya 2003; Cookson *et al.* 2006) as the process for leaf enlargement within this monocarpic species. Investigations showed considerable variation in leaf and inflorescence length over a season, and in addition, there was variation in the density of different kinds of hairs in the indumentums on the bracts and calyces over a season. Thus, it is suggested that this kind of study should be undertaken before using leaf size, inflorescence length and the indumentum as characters for taxonomic purposes, especially for monocarpic plants. This study does not necessarily suggest that the species described by Clarke are not worthy of recognition, but it brings the characters he has used in his key (i.e. the leaf and inflorescence length and possibly indumentuma of bracts and calyces) into some question.

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