www.czasopisma.pan.pl PAN www.journals.pan.pl

ACTA BIOLOGICA CRACOVIENSIA Series Botanica 54/1: 121–128, 2012
DOI: 10.2478/v10182-012-0013-z

PHENOMENA, BIOMETRICS AND CANOPY BEHAVIOR OF PRICKLY COMFREY (*SYMPHYTUM ASPERUM* LEP., BORAGINACEAE) IN A LONG-TERM EXPERIMENT

TADEUSZ ANISZEWSKI*

Research and Teaching Laboratory of Applied Botany, Biological Interactions and Ecological Engineering, Department of Biology, University of Eastern Finland, Joensuu Campus, P.O.Box 111, 80101 Joensuu, Finland

Received September 7, 2011; revision accepted May 22, 2012

Annual and interannual phenomena and canopy behavior of prickly comfrey (*Symphytum asperum* Lep.) were studied in a 10-year experiment with 25 measurement sessions during the growing season. The results confirm the importance of long-term experiments in studying plant phenomena, biometrics and behavior. Prickly comfrey produced a green canopy each year and growth started very early in spring. Maximum plant height was less than 160 cm. Annual phenomena (growth initiation, seedling phase, flower phase, seed phase, senescent phase), interannual phenomena (initiation and youth, reproduction, new generation formation, plant death) and two population cycles (colonization and expansion) were measured. The duration of annual development up to canopy death can be expressed as x+2x+3x+2x, where x is initial growth. The genetic structure and activity of prickly comfrey promotes generative development of the species. Its age can be measured over a single and several vegetation generations. The ability to change the angle of vertical stem growth after 9 weeks can be considered a functional behavior of prickly comfrey and part of its life strategy. The differences between the organs in the upper and lower parts are very considerable and should be taken into account in morphological descriptions of this species. The upper and lower stems and leaves showed differential growth. Both stem and leaves were densely setose. Old leaves were 3.8 times longer, 4 times broader and 2.4 times thicker than young leaves. Hairs were on average 3 times longer on old than on young leaves. Flowers had contact with pollinators making relatively long visits to them.

Key words: Biometrics, development, growth, long-term experiments, phenomenology, plant age, plant behavior, *Symphytum asperum*.

INTRODUCTION

Long-term experiments yield data crucial to understanding the life course of species in evolutionary biology, botany and ecology (Aniszewski, 2000, 2010; Rozen and Lenski, 2000; Dudley, 2007; Phillippe et al., 2007). The life behavior and genetic expression of perennial plant species cannot be explained well without such data. Long-term results are also needed in current scientific debate on basic questions concerning plant life (Garzon and Keijzer, 2011; He et al., 2011). Although plant behavior plays a vital role in processes of natural and managed habitats, plant functional dynamics are not understood well enough to predict plant performance in future climatic scenarios (Osmond et al., 2004). Crop resources cannot be exploited efficiently without a good understanding of the dynamics of plant functioning (Schurr et al., 2006). The behavior, biometrics and the phenomenal dynamics of perennial plants continue to be challenging research topics. At the Finnish Agricultural Research Center, in the 1980s we began long-term observations and measurements of some potentially useful plants and their populations (Aniszewski and Simojoki, 1984; Aniszewski, 1988) and have continued this line of research in our laboratory (Aniszewski, 2000, 2004, 2010; Aniszewski et al., 1996, 2001). Prickly comfrey (*Symphytum asperum* Lep. Boraginaceae) was included in our long-term laboratory studies in 1991, as no long-term data on its behavior and phenomenal dynamics were available. Prickly comfrey is well-known in Finland, mainly as a decorative plant in gardens and parks or as an escapee in semi-natural ditches and other damp places (Mossberg and Stenberg, 2005). It is one of the four *Symphytum*

*e-mail: Tadeusz.Aniszewski@joensuu.fi

PL ISSN 0001-5296

species currently found in Finland. It differs morphologically, genetically and chemotaxonomically from the widely distributed common comfrey (*Symphytum officinale* L.), less widely distributed Russian comfrey (*Symphytum* × *uplandicum* Nym.), and tuber comfrey (*Symphytum tuberosum* L.), a rare newcomer in southern Finland. Prickly comfrey (*Symphytum asperum* Lep.) differs from the other comfrey species in flower color and the sizes of organs, more vigorous growth (Mossberg and Stenberg, 2005), DNA, ploidy level and chemotaxonomic markers (Gadella and Kliphuis, 1975, 1978; Gadella et al., 1983; Jaarsma et al., 1989, 1990; Ozcan, 2010; Barbakadze et al., 2011). Among the comfreys mentioned, prickly comfrey has the smallest chromosome number (2n = 32) and contains echimidine alkaloid but not isobauerenol, which is typically found in common comfrey. Russian comfrey contains both of these alkaloids and is a cross hybrid between prickly and common comfrey (Jaarsma et al., 1989). Prickly comfrey (*Symphytum asperum* Lep.) is native to the Caucasus and Iran (Mossberg and Stenberg, 2005) but has been naturalized in many parts of Asia, Europe, America, Africa and Australia, especially in former British colonies (Tutin, 1956; Wade, 1958). The global distribution of this plant was driven by its economic potential in agriculture, horticulture and the pharmaceutical industry. In its native range of distribution, prickly comfrey has been used in folk medicine since ancient times. In the 18th century this plant was used as an ornamental in the gardens of Russian aristocrats, from where it spread to gardens in Europe. From the 19th century onward, prickly comfrey was known as a field plant cultivated mostly for animal fodder and as a promising silage plant, especially in England but also elsewhere in Europe. It is still cultivated for green manure and composting purposes in organic cultivation. Since 2003 the use of prickly comfrey in livestock feeding has been outlawed in the U.S.A. and many other countries. The strong bioactivity of its secondary compounds (pyrrolizidine alkaloids, allantoin and rosmarinic acid) have restricted prickly comfrey to medicinal uses and as fertilizer. Prickly comfrey is used in the Caucasus in folk medicine to treat wounds, ulcers and rheumatoid arthritis (Barbakadze et al., 2011). Prickly comfrey is now part of the global flora, occurring in natural and semi-natural areas on all continents. Research on the behavior of this plant should prove useful in predicting how climate change may affect biodiversity. As the published long-term data on this species are sparse, we chose to research the biometrics, behavior, and both annual and interannual phenomena of this plant in a series of long-term experiments, assessing the vigor of growth, typical canopy development and morphological traits of this plant in a boreal zone. The study addressed one basic question: What are the annual and interannual phenomena in the growth dynamics and canopy behavior of prickly comfrey?

MATERIAL AND METHODS

PLANT MATERIAL AND EXPERIMENTALLY CONSTRUCTED HABITAT

The ecotype and population history of the seeds of prickly comfrey (*Symphytum asperum* Lep.) harvested in 1990 and used in these long-term experiments were not known. The experiment was begun in 1991 in Botania, the botanical garden of the University of Joensuu, Finland (62°36'00''N, 29°45'50''E), employing standard procedures for plant acclimation and measurement, with some modifications. The experimental site was in the center of the botanical garden, in the research zone between a path and a wild area of trees and bushes. The plots were laid out and well demarcated on the soil surface in an east-west direction, with buffer plots on the easternmost and westernmost ends. The prickly comfrey seeds were sown over a 1 m^2 plot in 4 replicates and 2 buffer plots. The soil was tilled manually down to a depth of 30 cm. In each plot, 100 seeds were sown at 3 cm depth on May 15, 1991. The resulting vegetation was measured and observed over 10 years starting from 1992. Each year, after the final measurements, the plants were cut to 10 cm height from the soil surface. Sample and data analyses were done during 2002–2010. The experimental habitat (Botania) is located in the Finnish boreal zone, strongly influenced by the Gulf Stream from the Atlantic and by gentle southwestern winds, producing a climate 3–4°C warmer than other areas at the same northern latitude. The climate is continental with hot summers and cold winters. The soil in each plot was natural and typical garden soil with fine sand, sand, silt, clay and organic matter fractions (for details see Aniszewski, 2010). Soil pH in the plots was measured at the beginning of the experiment at three depth levels using pH indicators and a Batex IM 555 pH & mV meter. The characteristics of the experimentally constructed habitat for prickly comfrey are presented in Table 1.

MEASUREMENTS AND OBSERVATIONS

The plants were measured and observed 25 times per year for ten years on the same dates at weekly intervals. The observation period started on 15 April and ended 15 October of each year. The first measurement date was extremely early (weather changeable from frost and snow to spring warmth and humidity) and the last measurement day was

TABLE 1. Climate and trial characteristics

Parameter	Value/date
GP	160 d
ETS	1100°C
AAT	2°C
PSF	5 months
PSTGP	400 mm
ADFS	November 1
ADPS	November 15
LTASD	60–70 cm
PN	4
TNA	10^6 mm^2
DSGL	3×10^{-2} m
S_1pH	5.74±0.25
S_2pH	5.71±0.3
S_3pH	5.70±0.4

Abbreviations: GP – growing period; d – days; ETS – effective temperature sum on average; ATT – annual average temperature; PSF – period of soil frost; PSTGP – precipitation sum during thermal growing period; ADFS – average day of first snow; ADPS – average day of permanent snow; LTASD – long-term average snow depth on March 15; PN = plot number; TNA – total net area of each plot; DSGL – depth of soil growing layer; S_1 – soil depth level 0–10 cm; S_2 – soil depth level 11–20 cm; S_3 – soil depth level 21–30 cm

extremely late (weather changeable from autumn warmth to frost and snow). The type of growth exhibited by prickly comfrey was noted, and all cases of deviations in growth were recorded and measured. Qualitative observations were made with magnifying glasses (diameter 5 cm at 5× and 10 cm at 10×), and an ocular (20×) and a Zeiss Stemi DV4 stereomicroscope. Plant density, stem length, and diameter of lower stem (1 cm from soil surface) and upper stem (1 cm from flower at top) were also measured. Measurements were made with standard instruments to 1 mm accuracy, and with an electronic micrometer with an automatic reader (Digimatic) to within 1 μm. The experimental plant cover on each plot was measured.

PHENOMENA

Both annual and interannual phenomena in the development of prickly comfrey were observed and measured. The annual phenomena concern organ development during the experimental period each year, and the interannual phenomena concern interannual development. The annual phenomena were fixed and measured 25 times each year. More than 50% of the individuals in the trial population were needed to show the phenomena. The interannual phenomena relating to the age of individuals and the cyclic development of prickly comfrey were also checked by statistical analysis of the data collected.

CANOPY CONTACT WITH POLLINATORS

Canopy contact in the experimentally constructed habitat was studied by observations of plot populations. During four sunny days between the 9^{th} and 12^{th} measurements, contact with pollinators was measured at 11:00 a.m. for one hour by counting the pollinators visiting 4 individual plants selected at random for observations. All the insects visiting the plants and their duration of visits to single flowers were measured.

FUNCTIONAL GROWTH AND DATA ANALYSIS

The botanical data collected during the 10-year experimental period reflect the dynamic nature of the development of the prickly comfrey plot populations in the habitat constructed for this purpose, and were analyzed with a combination of methods aimed at quantifying the potential functionality of plant growth dynamics. The results of the statistical analyses were then considered in the light of relevant theoretical possibilities and explanations. The growth dynamics and forms of growth were linked and their functionality and the recorded phenomena. The collected data were analyzed and presented in graphs using SigmaPlot 11.0 for Windows. ANOVA and normality tests were applied as previously (Aniszewski et al., 2001).

RESULTS

GROWTH PARAMETERS

The following annual phenomena were observed each year (Fig. 1a): phase of initiation growth (1–3 weeks), seedling phase (1–8 weeks), flower phase (6–15 weeks), seed phase (13–21 weeks) and senescent phase (from week 19). During the experiment the flowering cycles of the whole population of prickly comfrey varied between measurement days 4 and 14, and the seed reproduction cycles between 14 and 21 weeks. Canopy death in the plant population started during the senescent phase from the 21^{st} measurement onwards (Fig. 1a). Interannual phenomena were determined as the population initiation and youth phase (1–9 weeks), reproduction phase (9–14 weeks), formation of plant new generation (14–20 weeks), and death of individuals (from week 20). The development of the prickly comfrey population over the 10 years showed the following population phases (Fig. 1b): plant initiation and youth (from 1 to 7 weeks, with individual variation up to 9 weeks), plant reproduction phase (from 9 to 14 weeks, with individual variation of 7 to 14 weeks), formation of plant new generation (from 14 to 20 weeks), and finally the plant death phase start-

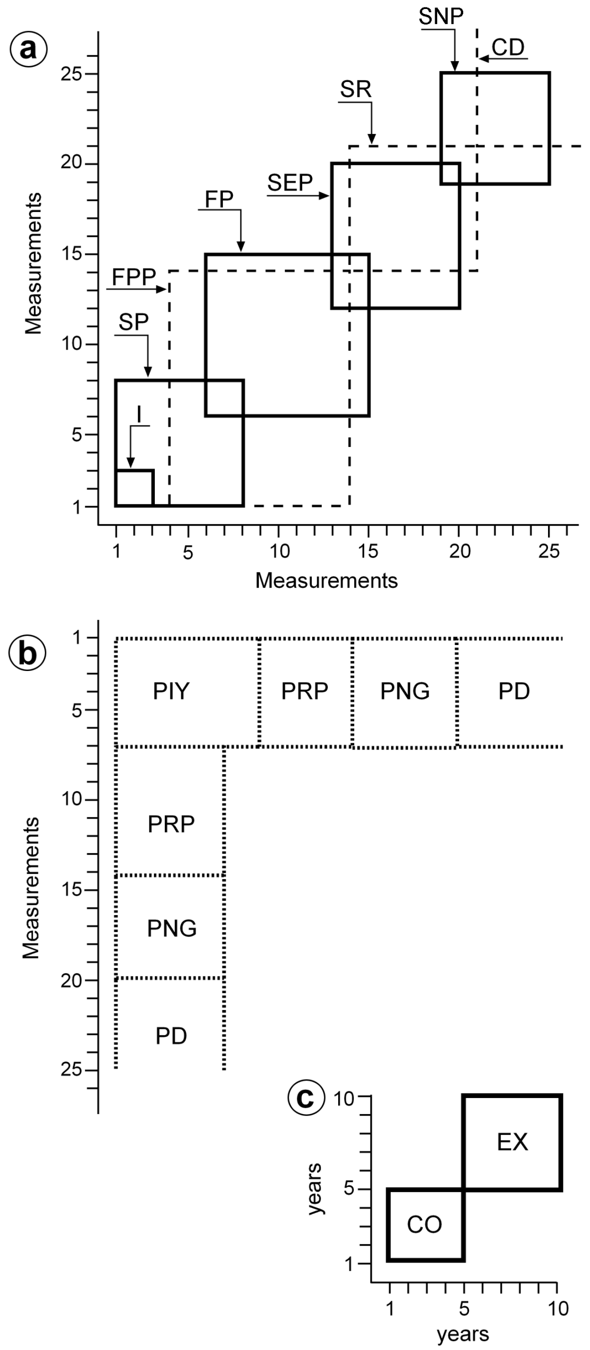

Fig. 1. Annual and interannual phenomena of prickly comfrey. (**a**) Annual phenomena and whole-population cycles. I – initial growth phase; SP – seedling phase; FP – flower phase; SEP – seed phase; SNP – senescent phase; FPP – flowering population phase; SR – seed reproduction; CD – canopy death in plant population, (**b**) Interannual phenomena and whole-population phases. PIY – plant initiation and youth phase; PRP – plant reproduction phase; PNG – formation of plant new generation; PD – plant death, (**c**) Interannual developmental cycles of prickly comfrey population. CO – colonization of plot space and area; EX – expansion in space and area.

ing from week 20 (Fig. 1b). The prickly comfrey population also had two clear interannual developmental cycles: colonization of plot space and area, and expansion in space and area. Each cycle lasted 5 years (Fig. 1c).

Plant density in the experimental plots was similar at all measurement times (Fig. 2a). Average density was less than 50 individuals per square meter and increased slowly from the beginning to the end of the annual growing periods, peaking at the 21st measurement. Standard deviations from the means were relatively large. The largest deviations were at the beginning of the annual growing period (Fig. 2a).

Plant growth started early, observed at the first measurement date (Fig. 2b). Vegetation height peaked at the 11th measurement and remained close to the peak values for 4 weeks (weeks 9–13). Plant height did not exceed 160 cm. The stems were no longer able to grow vertically and consequently plant height fell to less than 60 cm at the last measurement date (Fig. 2b). All the data were highly significant and the reduction in plant height was not influenced by any factor external to the experiment.

CANOPY COVER AND EXPANSION

The plant population established green vegetation each year. Mean canopy cover was 20% of total plot area and increased rapidly during the vegetation period (Fig. 2c). At the 9th measurement mean plot cover was more than 1 m^2 (100%), and close to 1.4 m^2 at the 25th measurement. Canopy extended over the plot borders as the angle of the main stem changed. In the first few measurements it was exactly 90°, with all plants showing vertical growth. At the 9th measurement, further growth of leaves and expansion of cover outside the plot borders was observed. The angle of the main stems ranged from 75° to 85°. At the 25th measurement the stem angle ranged from 45° to 70° in all directions across the plot. The standard deviations were highest during the beginning of the growing period (statistically significant relations between data) and lowest in the later stage of vegetation (data relations close to significance) (Fig. 2c).

STEM DATA

Stem diameter during the growth of prickly comfrey was observed (Figs. 2d,e). In both upper and lower parts of the stem, rapid mean growth in diameter was measured at the 9th measurement date. Lower stem diameter was 1.22±0.333 mm on average on the first measurement day, rising to 8.07±0.512 mm during the next 7 weeks (8th measurement). During the next week the lower stems grew rapidly to 15.47±1.827 mm. The lower stems were thickest, 18.48±0.471 mm, at the 16th measurement. Lower

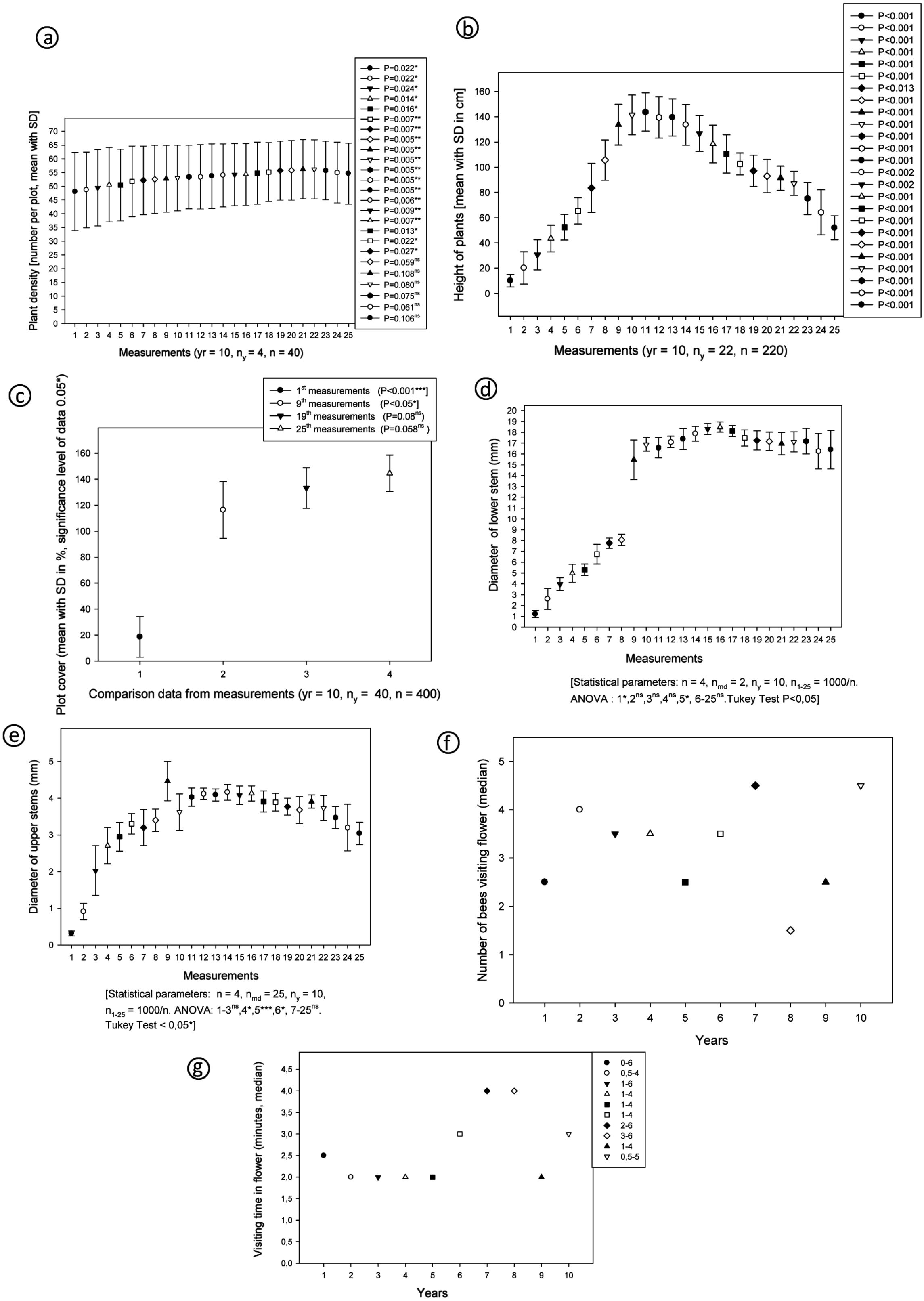

Fig. 2. Biometrics and canopy behavior of prickly comfrey. (**a**) Plant density, (**b**) Height of plants, (**c**) Plot cover, (**d**) Diameter of lower stem, (**e**) Diameter of upper stem, (**f**) Number of bees visiting flower, (**g**) Visiting time in flower.

TABLE 2. Morphological parameters of prickly comfrey during the experiment

Plant parameter	Mean (mm ±SD)
Length of 1st lower leaf	138.23±14.51
Width of 1st lower leaf	62.52±10.35
Thickness of 1st lower leaf	2.36± 0.74
Length of petiole of 1st lower leaf	53.21±9.90
Diameter of petiole of 1st lower leaf	7.07±1.0
Length of hairs on lower leaf	1.5±0.5
Length of 1st upper leaf	35.77±5.0
Width of 1st upper leaf	15.40±6.0
Thickness of 1st upper leaf	1.0±0.16
Length of petiole of 1st upper leaf	11.18±5.1
Diameter of petiole of 1st upper leaf	1.24±0.6
Length of hairs on upper leaf	0.5±0.2
Length of hairs on lower stem	3.0±1.5
Length of hairs on upper stem	0.9±1.0
Length of fully developed flower	16.79±2.0
Length of calyx	4.2±1.3
Length of corolla	13.3±1.4

stem diameter was 16.40±1.765 at the last measurement (Fig. 2d).

Mean upper stem diameter was 0.319±0.679 mm at the first measurement and 3.4±0.309 mm at the 8th measurement. The value was highest (4.47±0.538 mm) at the 9th measurement, decreasing thereafter to 3.04±0.303 mm at the last measurement (Fig. 2e).

MORPHOLOGICAL PARAMETERS

The leaves of prickly comfrey are ovate to oblong, with 138.2±14.51 mm average length, 62.52±10.35 mm width and 2.36±0.74 mm thickness in lower leaves. The corresponding measurements of top leaves were 35.77±5.0 mm, 15.40±6.0 mm and 1.0±0.16 mm (Tab. 2). The leaves are petiolate. The petiole of lower leaves was on average 53.21±9.90 mm long and 7.07±1.0 mm in diameter; the petiole of upper leaves was 11.8±5.1 mm long and 1.24±0.6 mm in diameter. The leaves are densely setose. The length of hairs was 1.5±0.5 mm on the lower leaf and 0.5±0.2 mm on the upper leaves. Aculeate hairs were also present on the stem. The hairs were 3.0±1.5 mm on the lower and 0.9±1.0 mm on the upper stems (Tab. 2). When fully developed, the flower was 16.79±2.0 mm long. Corolla length was 13.3±1.4 mm and calyx length 4.2±1.3 mm (Tab. 2).

Old leaves were on average 3.8 times longer, 4 times broader and 2.4 times thicker than young leaves (Tab. 2). Mean petiole length of old leaves was 4.7 times and mean diameter 5.7 times that of young leaves. Hairs grow on both sides of the leaves and on stems. Hairs on old leaves were on average 3 times longer than on young leaves. The length ratio of lower and upper stem hairs was 3.3. The corolla of the fully developed flower was 3.2 times longer than the calyx (Tab. 2).

ATTRACTION OF BEES TO FLOWERING CANOPY

Bees visited flowering plants on average 3 times per individual plant. The median during each year was from 0 to 5, with a maximum of 6 visits (Fig. 2f). The highest median was in the 7th and 10th years. Cases of no visits were observed only in the 1st and 9th years.

The median duration of a visit by a bee to one flower during the 10-year experiment was 2–4 minutes, with a minimum of 0.5 minutes and maximum of 6 minutes (Fig. 2g). The duration of visits was clearly higher in the 1st, 6th–8th and 10th experimental years. The medians and ranges for experimental years 2–5 and 9 were similar (Fig. 2i).

DISCUSSION

These results confirm the value of long-term experiments in the study of plant biometrics, behavior and botanical phenomenology. Prickly comfrey grew successfully in an experimentally constructed habitat for 10 years. The plant's canopy phenomena and behavior (individual, population, contact with pollinators) were observed and measured intra- and interannually. Prickly comfrey is a well-acclimatized species, as it was able to grow in an experimentally constructed habitat under natural growth conditions. Growth started very early in spring and full vegetation and organ development occurred during the short vegetation period with no signs of canopy damage, indicating that the plants had no difficulty in thriving under boreal zone climate. Climate is considered to be highly important in plant growth dynamics (Adler and Hillerislambers, 2008). The height of the population did not exceed 160 cm, which is less than the maximum length given for this species by Mossberg and Stenberg (2005) in their description of the flora of Scandinavia and by Tutin et al. (1972) in *Flora Europaea*, and the heights measured in this study are within the 50–200 cm range given by those same authors. The plant's density dynamics and canopy plot cover of over 100% by the population testify to its vigor and ability to compete for space in the habitat. Its tendency to cover and extend outside a plot is known and has also been reported for other species (Aniszewski, 2010). The deviation from vertical growth of the plants (i.e., from negative geotropism) in the second half of the vegetation period is a part of the behavioral strategy of the species. Individual plants take up more space outside the plot boundary, which reduces the distance between ripening seeds and the soil surface, assisting future seeding and guaranteeing local expansion of the population. The competitive outcomes of individual plants are ultimately determined by their behavior (Finnof and Tschirhart, 2009).

The behavior of the individual plant can be observed in the annual growth dynamics and interannual cyclicity of a plant population. Based on our results, variation in the duration of annual development up to the period of canopy death can be expressed mathematically as x+2x+3x+2x, where x is initial growth. This means that initial growth is of special importance for all canopy development and for the behavior and development of populations. The flowering phase lasts longer than any other phases. This suggests that prickly comfrey favors generative development.

Prickly comfrey shows generational development stages: initiation, youth and sexual (flower) stage, reproduction, and death. It also shows cross-generational development cycles at population level, such as colonization and expansion. However, the mechanism of annual (seasonal) cyclic and cross-generational ageing is not known. It may be that this mechanism is dependent not only on plant metabolism but also on structural and activity genes.

The plant's ability to change the angle of growth of the vertical stem after 9 weeks can be considered a functional behavior and part of the life strategy of prickly comfrey. The change in the growth angle of the canopy occurred at the same time as doubling of the diameter of the lower stem and a small increase in the diameter of the upper stem. These changes in the architecture of the plant ensured its stability during expansion. In accordance with the life strategy of the species, plants demonstrate adaptive behavior (Garzon and Keijzer, 2011): to maximize the amount of net energy that can be channeled to reproduction (Finnoff and Tschirhart, 2009), plants are able to make behavioral and morphological changes (Buswell et al., 2011).

The leaves of prickly comfrey are ovate to oblong, petiolate and densely setose (Tutin et al., 1972). The differences between the organs in the upper and lower parts are very considerable and should be taken into account in morphological descriptions of this species. The relations between the growth of organs in the upper and lower parts seem to remain constant.

According to our observations, prickly comfrey invites pollinators (bees), especially active during full flowering. Prickly comfrey has active contact with its surrounding habitat solely through its canopy behavior and pollinator activity. Bees frequently visited the flowers, and their visits were relatively long. Prickly comfrey is a very attractive plant for bees, as evidenced by observations of bees spending up to 6 minutes working on a single flower. During this time bees were in direct internal contact with the flower and with the pollen.

ACKNOWLEDGEMENTS

I thank Dr S.N. Drozdov for donating seeds for this research, and former Gardener-in-Chief Pekka Piironen for his participation at different stages of management of the long-term experiments. I also thank Aki Juhani Leinonen, Mervi Kupari, Veli-Pekka Pennanen, Minna Sinkkonen and Gäelle Gabriel for their practical contributions to this research during their work in my laboratory, and many of my former students, who enthusiastically assisted in this research. Kirsti Kyyrönen assisted in making Figure 1. Fruitful discussions with my colleagues and members of the International Research Team in Applied Botany are warmly acknowledged.

REFERENCES

ADLER PB, and HILLERISLAMBERS J. 2008. The influence of climate and species composition of the population dynamics of ten prairie forbs. *Ecology* 89: 3049–3060.

ANISZEWSKI T. 1988. Phenological study on trees, bushes and arable peat land. Agricultural Research Center, Jokioinen, Finland. *Bulletin* 2/88: 1–125. [In Finnish].

ANISZEWSKI T. 2000. Phenotypic parameters of elecampane (*Inula helenium*) in eastern Finland. *Aquilo Series Botanica* 38: 13–28.

ANISZEWSKI T. 2004. Legume species that have breeding potential for NE Europe. *Science of Legumes* 6: 256–265.

ANISZEWSKI T. 2010. Canopy behavior of three milkvetch (*Astragalus*) species in acclimation to a new habitat. *Acta Biologica Cracoviensia Series Botanica* 52/1: 45–54.

ANISZEWSKI T, DROZDOV SN, KHOLOPTSEVA ES, and MIHKIEV AI. 1996. Botanical characteristics and phenological development of *Galega orientalis* Lam. in the primeval forest zone of eastern Fennoscandia. *Aquilo Series Botanica* 36: 21–26.

ANISZEWSKI T, KUPARI MH, and LEINONEN AJ. 2001. Seed number, seed size and seed diversity in Washington lupine (*Lupinus polyphyllus* Lindl.). *Annals of Botany* 87: 77–81.

ANISZEWSKI T, and SIMOJOKI P. 1984. Quantity and vigour of weed seeds in some ARC rotation experimental areas. Review and analysis of soil samples from three ARC research stations. Agricultural Research Centre. Jokioinen, Finland. *Bulletin* 22/84: 1–38. [In Finnish].

BARBAKADZE VV, MULKIDZHANYAN KG, MERLANI MI, GOGILASHVILI LM, AMIRANASHVILI LSA, and SHABURISHVILI EK. 2011. Extraction, composition and the antioxidant and anticomplement activities of high molecular weight fractions from leaves of *Symphytum asperum* and *S. caucasicum*. *Pharmaceutical Chemistry Journal* 44:11: 604–607.

BUSWELL JM, MOLES AT, and HARTLEY S. 2011. Is rapid evolution common in introduced plant species? *Journal of Ecology* 29: 214–224.

DUDLEY JW. 2007. From Means to QTL: The Illinois long-term selection experiment as a case study in quantitative genetics. *Crop Science* 47 (S3): S20–S31.

FINNOFF D, and TSCHIRHART J. 2009. Plant competition and exclusion with optimizing individuals. *Journal of Theoretical Biology* 261(2): 227–237.

GADELLA TWJ, and KLIPHUIS E. 1975. Cytotaxonomic studies in Genus *Symphytum*. 7. Some hybrids between *Symphytum asperum* Lepech and *Symphytum officinale* L. in Denmark. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen Series C – Biological and Medical Sciences* 78:182–188.

GADELLA TWJ, and KLIPHUIS E. 1978. Cytotaxonomic studies in Genus *Symphytum*. 8. Chromosome numbers and classification of 10 European species. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen Series C – Biological and Medical Sciences* 81: 162–172.

GADELLA TWJ, KLIPHUIS E, and HUIZING HJ. 1983. Cyto- and chemotaxonomical studies in the sections *Officinalia* and *Coerulea* of the genus *Symphytum*. *Botanica Helvetica* 93: 169–192.

GARZON CP, and KEIJZER F. 2011. Adaptive behavior, root-brains, and minimal cognition. *Adaptive Behavior* 19:155–171.

HE GM, ELLING AA, DENG XW, MERCHANT SS, BRIGGS WR, and ORT D. 2011. The epigene and plant development. *Annual Review of Plant Biology* 62: 411–435.

JAARSMA TA, LOMANNS E, GADELLA TWJ, and MALINGRE TM. 1989. Chemotaxonomy of the *Symphytum officinale* agg. (*Boraginaceae*). *Plant Systematics and Evolution* 167: 113–127.

JAARSMA TA, LOHMANNS E, HENDRIKS H, GADELLA TWJ, and MALINGRE TM. 1990. Chemo- and karyotaxonomic studies on some rhizomatous species of the genus *Symphytum* (*Boraginaceae*). *Plant Systematics and Evolution* 169: 31–39.

MOSSBERG B, and STENBERG L. 2005. *Grand Guide to Northern Plants*, 928. Tammi, Helsinki. [In Finnish].

OSMOND CB, ANANYEV G, BERRY J, LANGDOM C, KOLBERG Z, LIN G, MONSON R, NICHOL CJ, RASCHER U, SCHURR U, SMITH S, and YAKIR D. 2004. Changing the way we think about global change research: scaling up in experimental ecosystem science. *Global Change Biology* 10: 393–407.

OZCAN T. 2010. Differentiation of *Symphytum* species using RAPD and seed fatty acid patterns. *Natural Product Communications* 5: 587–596.

PHILLIPPE N, CROZAT R, LENSKI RE, and SCHNEIDER D. 2007. Evolution of global regulatory networks during a long-term experiment with *Escherichia coli*. *BioEssays* 29: 846–860.

ROZEN DE, and LENSKI RE. 2000. Long-term experimental evolution in *Escherichia coli*. VIII. Dynamics of balanced polymorphism. *American Naturalist* 155: 24–35.

SCHURR U, WALTER A, and RASCHER U. 2006. Functional dynamics of plant growth and photosynthesis – from steady-state to dynamics. *Plant Cell and Environment* 29: 340–352.

TUTIN TG. 1956. The genus *Symphytum* in Britain. *Watsonia* 3: 280–281.

TUTIN TG, HEYWOOD VH, BURGES NA, MOORE DM, VALENTINE DH, WALTERS DH, and WEBB DA. 1972. *Flora Europea*, vol. 3, 104–105. University Press, Cambridge.

WADE AE. 1958. The history of *Symphytum asperum* Lepech. and *S.* × *uplandicum* Nyman in Britain. *Watsonia* 4: 117–118.