

POLISH JOURNAL OF ECOLOGY (Pol. J. Ecol.)	55	1	49-55	2007
--	----	---	-------	------

Regular research paper

Anna GAZDA¹, Jerzy SZWAGRZYK¹, Hilde NYBOM², Gun WERLEMARK²,

¹Department of Forest Botany and Nature Conservation, Agricultural University,
al. 29-Listopada 46, 31-425 Kraków, Poland, rlgazda@cyf-kr.edu.pl;

²Department of Crop Science, Swedish University of Agricultural Sciences, Balsgard, Fjalkestads
123-1, 291 94 Kristianstad, Sweden

MORPHOLOGICAL AND GENETICAL VARIABILITY OF *RUBUS HIRTUS* (WALDST. & KITT.) PLANTS UNDER PARTLY OPEN FOREST CANOPY

ABSTRACT: Blackberries growing in forests regenerate mainly in a vegetative way; however, detailed studies have shown substantial variability in performance of *Rubus* individuals. The main problem is if the differences among plants are genetically based, or are due to site heterogeneity? This study was aimed at determining 1) how large are the differences in performance of individual plants and how persistent they are during consecutive years, 2) how much of that variability can be explained by the local environmental conditions, especially the intensity of competition among plants, and whether the performance of individual plants is related to their genetic characteristics. In a partially cut mature beech forest in Western Carpathians 35 randomly chosen individuals of *Rubus hirtus* (Waldst. & Kitt.) were tagged and measured each year for 9 years. Ten largest and ten smallest individuals were selected for genetic analysis. Mean size of individual plants differed between consecutive growing seasons, but the overall size ranking was quite consistent throughout the entire period of study. Analysis of environmental variables: relative light intensity and local abundance of potential competitors explained about 24% of the differences in individual plant performance. In the genetic analysis the five primers were applied to analyse all 20 samples. The five primers produced 10 polymorphic bands, showing a high variability. Only a few samples displayed identical band patterns, indicating either vegetative propagation

or apomictic origin. Distribution of the results of Spearman's rank correlation of plant size among genetically related individuals did not differ significantly from the distribution of rank correlation coefficients in the entire sample. In the analysed population genetic affinity did not seem to correlate with the performance of *Rubus hirtus*.

KEY WORDS: clonal plants, population structure, vegetative reproduction

1. INTRODUCTION

Plants respond to periodic changes in resource availability in term of their growth, morphology and reproduction (Anderson *et al.* 1969, Pitelka *et al.* 1980). Especially clonal plants are well adapted to variation in resource availability in a patchy environment, such as a forest floor, where resources could be distributed very unevenly (Pancer-Koteja *et al.* 1998). Because of the reduced sexual recruitment, certain genotypes may be much more successful than others. These dominant genotypes may displace those that are less competitive during relatively short time. Clonal diversity thus may decrease, and monogenic stands may be established (Eriksson and Bremer 1993, Amsellem *et al.* 2000). Genetic variation

within apomictic *Rubus* species is relatively low (Nybom and Kraft 1995, Kraft *et al.* 1996). Because of that, *Rubus* is believed to have low genetic variability at the local scale. Despite numerous studies on taxonomy and biology of *Rubus* (Alice and Small 1999, Lambrecht-McDowell, Radosevich 2005, Taylor 2005), several questions remain open. Previous studies showed substantial variability in performance of individual *Rubus* plants growing under canopy gaps: especially the number of vegetative offspring produced by individual plant was highly variable among individuals (Pancer-Koteja *et al.* 1998). Measurements conducted on *Rubus hirtus* growing under a close canopy of mature beech forest (Gazda 2000) showed little variation in size of individual plants, very low mortality rates, no flowering and almost no tip-rooting. From previous studies (Pancer-Koteja *et al.* 1998) it was known, that immediately after the opening of forest canopy growth rate and size of individual plants increase and become more variable within population, flowering is frequent, and tip-rooting is a common phenomenon in all canes longer than 0.5 m. Thus we expected one of two scenarios: either the blackberries growing close to one another are genetically similar, and all the differences in their relative performance after canopy opening are due to environmental heterogeneity, or blackberries display some genetic variability, which influences their performance, and small genetical differences are amplified by environmental heterogeneity and by competition, creating a clear division into a group of well-growing and poorly-growing individuals.

Most studies on dynamics of population of clonal plants have focused on the ramet level (Harper 1977, Crawley 1990) mainly because genets are difficult to identify in the study area. In this paper we present 9-years series of field observations with genet identification by a molecular technique on *Rubus hirtus* as a clonal plant. The study was aimed at determining how large are the differences in performance of individual plants and how persistent are they during consecutive years. We also tried to find out how much of that variability can be explained by the local environmental conditions, especially the intensity of competition among plants, and whether

the performance of individual plants is related to their genetic characteristics.

2. MATERIAL AND METHODS

Investigations were carried out in a mature beech stand in the Krynica Experiment Forest (49°25'N, 20°53'E), southern Poland, in years 1993–2001. The detailed description of geology, soil, plant communities and climate in this site is given by Szwagrzyk *et al.* (2001). Our permanent plot ca. 700 m² was established in September 1993, under a loose cover of trees (after a partial cutting in winter 1992/1993). In the beginning of this study plants were exposed to a light intensity of approximately 76% of full sunlight. Due to crown expansion of beech trees, the illumination of the forest floor has gradually increased to 69% in 1994 and to 48% in 2000.

Blackberry (*Rubus hirtus* Waldst. & Kitt.) is one of the most common plants of the forest floor in mixed and deciduous forests of southern Poland. For several decades it has been increasing in abundance, and the partial-cutting system employed in forest management is widely believed to be the main reason for expansion. *Rubus hirtus* is a clonal plant with one or a few shoots growing horizontally in various directions; it reproduces vegetatively by tip-rooting. *Rubus* has several interesting biological features; first, it can survive for many years in deep shade by producing single, short shoots which do not tip-root (Gazda 1992). When the availability of light on the forest floor increases as a result of natural disturbance or partial cutting of forest stand, *R. hirtus* can expand rapidly by producing very long shoots every year, each of them tip-rooting. The rate of expansion of *Rubus* and its relation to the amount of light are quite well documented (Pancer-Koteja *et al.* 1998). Flowering and fruiting in fast-growing individuals is quite common (Gazda 2000), although flowers are believed to be mostly apomictic. *Rubus* seedlings are observed rather seldom (Pancer-Koteja *et al.* 1998, Gazda 2000).

Within the study area, 35 ramets of *Rubus* were chosen randomly in September 1993. At first the distribution of *Rubus hirtus* “individuals” (= ramets) was mapped and then they were measured, tagged and

monitored for four consecutive years. Cane length, number of inflorescences, the number of tip-rooting canes, and the number of new (established) ramets were recorded for each individual every year. In June 1999 we divided the tagged blackberries on the basis of their sizes (total length of all primocanes) attained during four years of study into three groups: 10 large individuals, 15 medium-sized and 10 small ones.

Since the year 1999 we have measured, tagged and monitored only the 20 individuals chosen for genetic study: 10 large individuals and 10 small ones. To estimate the level of competition for resources we determined the density of rooting points of neighbouring blackberries within a circle of the size of 0.5 m², and we estimated the percentage of cover of *Rubus* and the cover of other plant species within the same circle. Every year we labelled canes which were grazed by animals, so we were able to count how many times during our study every individual was disturbed by deer browsing.

The relative light intensities at the rooting point of each individual were determined in the year 2000 using the diazo-paper method (Friend 1961, George and Bazzaz 1999). In the calculations we used the measure of shading, calculated as the difference between full sunlight and the relative light intensity estimated by diazo-paper method.

For the genetic study we focused on the large and small individuals. For each plant, five youngest leaves were collected. Genomic DNA from the leaves was extracted according to the method of Nybom and Schaal (1990) from a total of 20 samples (Nos. 5, 15, 16, 19, 23, 28, 29, 31, 33, 34, 6, 8, 9, 11, 14, 20, 22, 27, 32, 35). PCR reactions for the RAPD analysis were performed in 25 µl, containing appr. 20 ng of DNA, 1X reaction buffer IV (Advanced Biotechnologies), 1.5mM MgCl₂ (Advanced Biotechnologies), 0.6 µl primer (Operon Tech. Inc.), 0.2 mM PCR Nucleotide Mix (de la Roche) and 1.0 unit Taq DNA Polymerase (Advanced Biotechnologies). The thermal cycler MJ Research was programmed for 5 min initial denaturation at 94°C, followed by amplification for 40 cycles at 1 min of 94°C, 1 min at 36°C and 2 min at 72°C and 7 min extension time at 72°C. The fragments were separated on a 1.8% agarose gel containing

ethidium bromide and using TPE buffer, and subsequently photographed under UV-light. The bands were scored manually for presence or absence of bands.

Computations were made using the program S-Plus 2000-trial, MathSoft. Multiple regression analysis was employed for relating the performance of individual plants to environmental variables, as: local light intensity, local density of potential competitors (number of rooting points within a 0.5 m² circle) and local cover of *Rubus*. Kendall's test of concordance was used to compare size rankings (to evaluate, how consistent are the size rankings in consecutive years of study). The Spearman's rank correlation of plant size (total length of primocanes produced by one individual) was calculated for the entire sample. Then the subset of correlations calculated for genetically similar individuals was separated, and the Kolmogorov-Smirnov two-sample test was used for comparing the distribution of Spearman's rank coefficients for the whole group with the distribution of rank coefficients calculated for genetically related individuals.

3. RESULTS

In the beginning of this study dimensions (total length of all primocanes attained during one growing season) of individual *Rubus* plants were relatively small (29.4 cm ± 34.45). Only 15% of individuals were able to produce more than 50 cm of primocanes. In the next four years the average size of individual has increased (Fig. 1), but the index of variability has decreased substantially. The mean size of *Rubus* plant differed between consecutive growing seasons. Every year, in our population most of individuals (60%) grew as small ones; they produced less than average total length of primocanes per individuals. Only every third (35%) individuals grew more often as large (they produced primocanes equal or longer than average) than small ones. But from "ramets point of view" only every fourth individual behaved in the same way from year to year: 20% grew as small ones and 5% as large ones. Next 25% of individuals only occasionally (once or twice per nine years of observation) grew as large ones; and another 20% of individuals grew

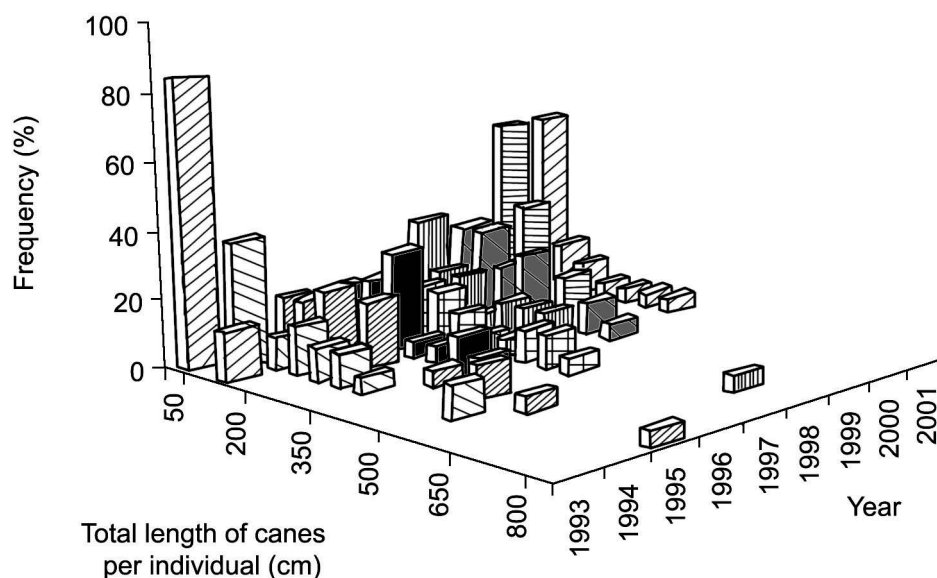


Fig. 1. Frequency histogram of total length of primocanes per individual.

Table 1. Summary of results of RAPD analysis (primers which were used to screen for polymorphism among the samples).

Primer ID	Sequence	No. polymorphic bands
OPA-7	GAAACGGGTG	1
OPA-8	GTGACGTAGG	2
OPG-6	GTGCCTAACC	3
OPG-14	GGATGAGACC	2
OPG-17	ACGACCGACA	2

occasionally as small ones. Rest of them seemed to grow without any clear pattern. The results of Kendall's test of concordance ($W = 0.471, P = 0.000$) indicated, that the size ranking of *Rubus hirtus* plants was consistent throughout the entire period of study.

Vegetative reproduction by tip-rooting in the first year of study was very infrequent (10%). Almost every plant (95%) tried to regenerate vegetatively in the next years, and 90% of them produced new ramets. No blackberries flowered during the first season. In the next years 75% individuals produced floricanes with inflorescences. The number of fruits produced by plants varied from 2 to 112 complex fruits per individual.

Thirty primers were used to screen for polymorphism among the samples 6, 8, 15 and 29 and five of them which produced

clear and reproducible bands were then applied to analyse all 20 samples. Sample no 28 was excluded from further analyses due to insufficient DNA. The five primers produced 10 polymorphic bands (Table 1), showing very high variability among the remaining 19 samples (Fig. 2). Some samples displayed identical band patterns, indicating either vegetative propagation or being of apomictic origin. Samples 5, 31 and 34 showed identical band pattern and separated with only one band from the identical samples 9 and 32. Samples 23 and 35 also showed identical band pattern and were separated with one band from sample 29, and samples 6, 15 and 16 also displayed identical band pattern. Also, samples 11 and 14 were only separated by one band from each other. In total, the samples were separated into 5 groups con-

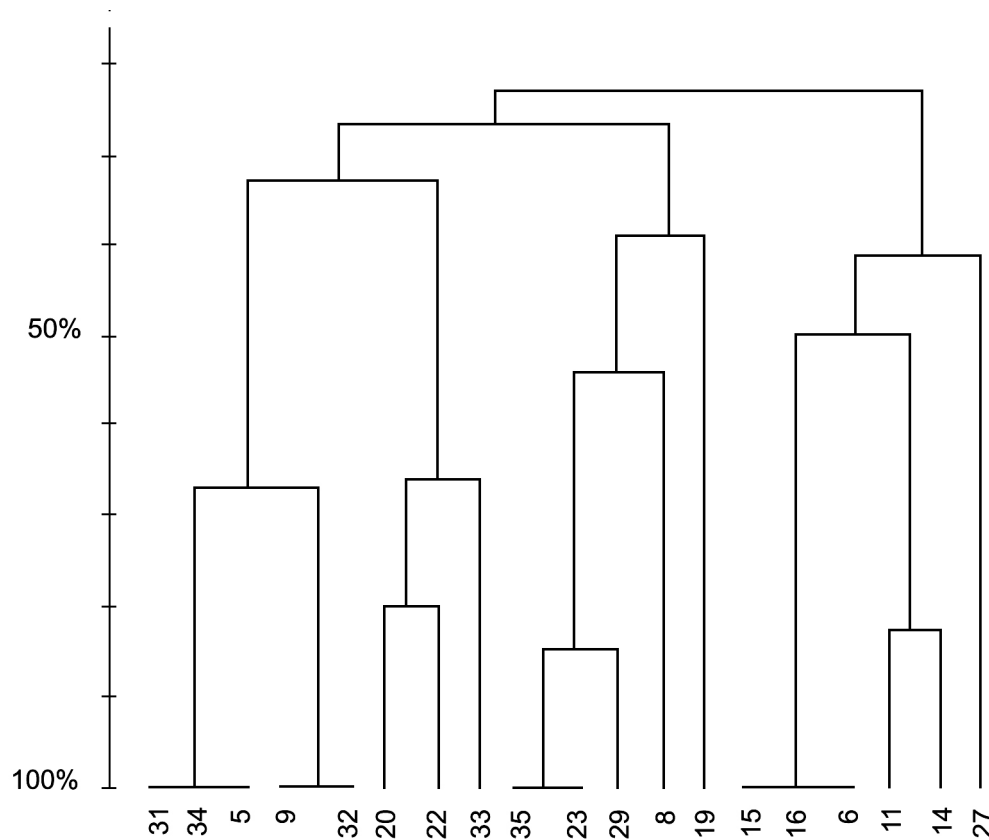


Fig. 2. Genetic affinities among the 19 samples of *Rubus hirtus*. (The dendrogram based on the result of 10 RAPD markers. On the horizontal line you start at 100% it does mean absolute agreement among the samples, we have termed it 'apomicts'; and then continue from left to right 90, 80....20%; On the vertical line there are label of samples = ramets).

sisting of samples 5, 9, 31, 32 and 34 in one group, samples 20, 22 and 33 in the second, 23, 29 and 35 in the third group, samples 6, 15 and 16 in the fourth, and samples 11 and 14 in the fifth (Fig. 2). Each group were separated by three bands or more from each other. In addition, there were three samples (8, 19 and 27) which did not belong to any of these groups and seemed to be quite separated from them and from each other.

Multiple regression analysis showed, that environmental variables (relative light intensity, local density of rooting points of *Rubus hirtus* and local percentage of blackberries cover) explained 23.7% of the total variation in plant size ($P = 0.000$). However, very strong positive correlation was found between the size of individual *Rubus* plant and intensity of deer browsing ($r = 0.899$, $P = 0.000$), measured as a number of browsing episodes during the 9 years of study.

Distribution of the results of Spearman's rank correlation of plant size among genetically related individuals, analyzed with the Kolmogorov-Smirnov two sample test, did not differ significantly from the distribution of rank correlation coefficients in the entire sample ($DN = 0.171$, $P = 0.635$).

4. DISCUSSION

The results of our study are surprising in several ways. First, the local genetical variability in *Rubus hirtus* turned out to be relatively large, which we did not expect at all. During our studies there were distinguished 9 genets within 20 blackberry 'individuals'. We expected very low genetic variability because of the absence of seedlings coming from sexual reproduction. In Poland, intraspecific variation seems to be a little bit higher than in other *Rubus* species studied in Scandina-

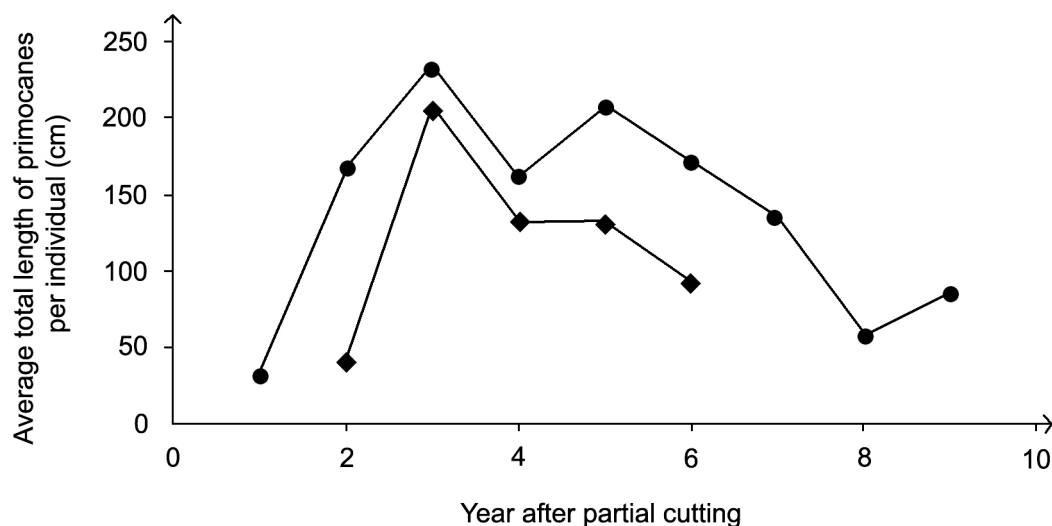


Fig. 3. Reaction of *Rubus hirtus* to canopy opening. (solid square – from Pancer-Koteja *et al.* 1998, solid circle – this study).

via and Germany (Gustafsson 1943, Weber 1972, Nybom and Kraft 1995, Kraft *et al.* 1996, Nybom and Bartish 2000), but we do not know if it is due to contemporary gene flow, somatic mutation or just some historical reasons.

Second, as shown by the comparison of the rank correlation coefficients, genetic make-up does not seem to govern the performance of *Rubus*, defined either by plant size, or by number of vegetative offspring, or by number of produced complex fruits. This result suggests, that the lack of strong relationship between genetics and competitive ability of individual plants can contribute to the maintaining of high genetic variability in a population, where most of the offspring is produced in a vegetative way.

Third, despite the fact, that the local environment seems quite constant, the length of *Rubus* canes changes from year to year; certainly, there is a general pattern of rapid increase in cane length after gap creation, and then a gradual decline in mean plant size (Fig. 3), but apart from that there is a lot of year-to-year variability. Deer browsing can be a significant factor influencing growth patterns in *Rubus*, as the browsing intensity is relatively high, and there is a strong correlation between plant size and the probability of being browsed.

Some of our results are less surprising. Two factors influencing plant growth – local light intensity and local abundance of potential competitors – explained together almost 1/4 of the total variation in individual plant performance. But we still do not know what causes the rest of the variability. As the size ranks are more-or-less consistent in time, it is possible that differences in plant performance are produced by the heterogeneity of below-ground resources.

We conclude, that in the analysed population genetical affinity did not seem to correlate with the performance of *Rubus hirtus*, and that a large part of variation in individual plant performance is yet to be explained.

ACKNOWLEDGEMENTS: We thank Anne Tolvanen, Pia Mutikainen, Pirkko Siikamäki and Juha Tuomi for their comments on the earlier version of the manuscript. We are grateful to Jan Bodziarczyk, Janusz Szewczyk and Tomasz Skrzydłowski for their assistance in the collecting field data.

5. REFERENCES

- Alice L.A., Small C.S. 1999 – Phylogeny of *Rubus* (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences – *Am. J. Bot.* 86: 81–97.

- Amsellem L., Noyer J.L., Le Bourgeois T., Hossaert-McKey M. 2000 – Comparison of genetic diversity of the invasive weed *Rubus alceifolius* Poir. (Rosaceae) in its native range and in areas of introduction, using amplified fragment length polymorphism (AFLP) markers – *Mol. Ecol.* 9: 433–455.
- Anderson R.C., Loucks O.L., Swain A.M. 1969 – Herbaceous response to canopy cover, light intensity and throughfall precipitation in coniferous forests – *Ecology*, 50: 255–263.
- Crawley M.J. 1990 – The population dynamics of plants – *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 330: 125–140.
- Eriksson O., Bremer B. 1993 – Genet dynamics of the clonal plant *Rubus saxatilis* – *J. Ecol.* 81: 533–542.
- Friend D.T.C. 1961 – A Simple Method of Measuring Integrated Light Values in the Field – *Ecology*, 42: 577–580.
- Gazda A. 1992 – Warunki występowania jeżyny gruczołowej *Rubus hirtus* (W. K.) w rezerwacie ścisłym “Dolina Łopusznej” w Gorczańskim Parku Narodowym [Conditions of occurrence of the blackberry *Rubus hirtus* (W. K.) in the strict reserve “Dolina Łopusznej” in the Gorce National Park] – *Par. Nar. Rez. Przyr.* 11: 105–117 (in Polish).
- Gazda A. 2000 – Response of *Rubus hirtus* to canopy gaps: results of long-term studies in Western Carpathians (In: *Plant population Biology VI*, Ed. P. Elias) – SEKOS, Bratislava-Nitra, pp. 120–125.
- George L.O., Bazzaz F.A. 1999 – The fern understory as an ecological filter: Emergence and establishment of canopy-tree seedlings – *Ecology*, 80: 833–845.
- Gustafsson A. 1943 – The Genesis of the European Blackberry Flora – *Lunds Univ. Arsskrift* 39 (6): 1–200.
- Harper J.L. 1977 – The population biology of plants – Academic Press, London, UK.
- Kraft T., Nybom H., Werlemark G. 1996 – DNA fingerprint variation in some blackberry species (*Rubus* subg. *rubus*, Rosaceae) – *Plant Syst. Evol.* 199: 93–108.
- Lambrecht-McDowell S.C., Radosevich S.R. 2005 – Population demographics and trade-offs to reproduction of an invasive and non-invasive species of *rubus* – *Biological Invasions*, 7: 281 – 295
- Nybom H., Bartish I.V. 2000 – Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants – *Persp. Plant Ecol. Evol. Syst.* 3/2: 93–114
- Nybom H., Kraft T. 1995 – Application of DNA fingerprinting to the taxonomy of European blackberry species – *Electrophoresis*, 16: 1731–1735.
- Nybom H., Schaal B.A. 1990 – DNA “fingerprints” reveal genotypic distributions in natural populations of blackberries and raspberries (*Rubus*, Rosaceae) – *Am. J. Bot.* 77: 883–888.
- Pancer-Koteja E., Szwagrzyk J., Bodziarczyk J. 1998 – Small scale spatial pattern and size structure of *Rubus hirtus* in a canopy gap – *J. Veg. Sci.* 9: 755–762.
- Pitelka L.F., Stanton D.S., Peckenham M.O. 1980 – Effects of light and density on resource allocation in a forest herb *Aster acuminatus* (Compositae) – *Am. J. Bot.* 67: 942–948.
- Szwagrzyk J., Szewczyk J., Bodziarczyk J. 2001 – Dynamics of seedling banks in a beech forest: results of a 10-year study on germination, growth and survival – *For. Ecol. Manage.* 141: 237–250.
- Taylor K. 2005 – Biological Flora of the British Isles: *Rubus vestitus* Weihe – *J. Ecol.* 93: 1249–1262.
- Weber H. E. 1972 – Die Gattung *Rubus* (Rosaceae) im nordwestlichen Europa – *Phanerogamarum Monogr.* 7, 504 pp.

(Received after revising August 2006)