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Searching for Weed Biocontrol Agents—When to Move on?

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'Classical' biological control for an exotic weed requires time-consuming and expensive surveys for natural enemies in the weed's native range. We advocate the use of rarefaction curves to improve survey efficiency, i.e. to determine the minimum sampling effort for discovering most of the potential control agents actually occurring in the weed's native range. Rarefaction (dilution) curves can be used to estimate the number of herbivore species expected on a given number of plants, at sampling sites or regions, using presence/absence data and species frequencies. An analysis of the shape of the rarefaction curves will therefore indicate (a) which sites possibly contain more undiscovered herbivore species and (b) whether sampling new sites is more likely to reveal further herbivores. This approach is illustrated with two case studies of insect surveys, of root and flower head feeders on Centaurea maculosa *(Asteraceae) in Europe and for flower head feeders of various Asteraceae in Brazil. Finally, we consider consequences of combining this approach with focussed searches in the centre of endemism and propose a general survey protocol for natural enemies associated with a host plant in its native range.*

Keywords: *biological control of weeds, field survey, sampling procedure, species accumulation curve, rarefaction curve,* Centaurea maculosa

INTRODUCTION

'Classical' biological control of an exotic weed inevitably requires time-consuming and often expensive surveys for natural enemies in the weed's native area (Schroeder, 1983; Müller, 1990). What is the most effective way of carrying out such surveys? In particular, within regions previously identified on other grounds (e.g. climatic or political): (a) where should the survey start and (b) when should the survey team move from one site to another, or from one region to another?

The primary aim of this study is to suggest a simple approach—based on theoretical considerations—to determine the minimum sampling effort required to discover most of the potential control agents in a survey region.

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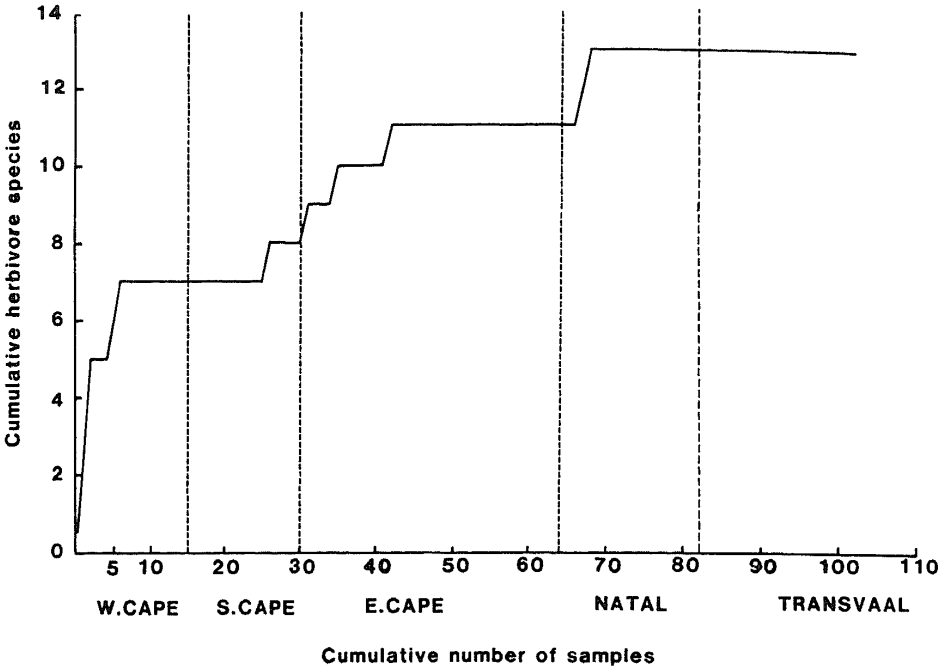


FIGURE 1. The cumulative number of species of bracken-feeding arthropods found with increasing number of samples in different regions in South Africa. A sample consists of at least 20 fronds examined at one site (from Compton *et al.*, 1989).

It is a familiar observation that repeated sampling at one locality, or at localities within a region, brings diminishing returns in terms of the rate of discovery of new, potential control agents. This is illustrated in Figure 1—from Compton *et al.* (1989)—relating to searching for biocontrol agents on bracken in South Africa for use in the UK (Lawton, 1988, 1990). It shows asymptotic species accumulation curves for insects on bracken within geographical areas of South Africa, temporarily accelerating rates of discovery of new species with each move to a new region, and an asymptotically declining rate in the species-accumulation curve for the country as a whole. The cumulative number of species showed no further increase after 70 of a total of 102 sites had been sampled.

How can these familiar observations be formalized to improve the effectiveness of searches for control agents? A random search throughout the weed's native range, or various parts of the exotic range, is the only suggestion available to practitioners of biological weed control (Schroeder, 1983). No further guidelines exist to aid decisions about how many plant individuals per site should be investigated, or how many sites should be visited to assess the regional species pool. In addition, once an initial field survey has been conducted, it would be interesting to know where further samples should be collected to efficiently increase the list of potential control agents.

In this paper, we advocate the use of rarefaction curves to improve survey efficiency. First, we explain rarefaction curves and discuss how these curves can express features of different insect–plant systems expected from theoretical assumptions. The approach is then illustrated using data from two insect surveys, one on Asteraceae in Brazil and the other on knapweeds in Europe. Finally, we consider consequences of combining this approach with focussed searches in the centre of endemism and suggest a basic protocol for programming an extensive survey for natural enemies in the weed's native range.

RAREFACTION CURVES

Several attempts have been made to use 'collector's curves' (number of species plotted against sampling effort, such as the number of samples, observation time, transect length

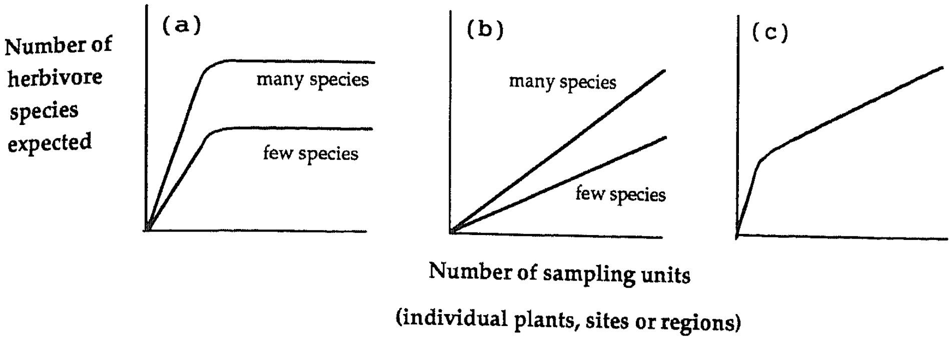


FIGURE 2. Theoretical relationships between increasing number of sampling units and the number of herbivore species expected (= rarefaction curves) for herbivore species differing in abundance and distribution. (a) Common and widespread herbivore species; (b) rare and localized herbivore species; (c) few common and many rare species of herbivores.

(Krebs, 1989)—Figure 1 is one such example) to obtain estimates of total species number in a community or region. If the curve has clearly levelled off, it provides evidence that all species have been recorded. However, if the curve is still rising, the estimation of its asymptote, which corresponds to the 'true' species total, is a difficult problem. Purely graphical extrapolations are scale-dependent and therefore unreliable (Hopkins, 1955). Analytical methods depend on the often arbitrary choice of a theoretical species-abundance distribution (May, 1975; Southwood, 1978; McGuinness, 1984). Therefore, the usual collector's curve, plotted in the original sampling sequence, is of limited value for present purposes. Also, stochastic sampling effects frequently make it difficult to decide whether or not cumulative collector's curves have or have not reached an asymptote. Rarefaction curves average the data and remove stochastic sampling effects.

Rarefaction curves were first proposed to compare species richness between different sized samples. From the relative abundance of each species within a single large sample, expected numbers of species can be calculated for any smaller sample size (Sanders, 1968; Hurlbert, 1971; Magurran, 1988). Rarefaction curves can also be obtained from species occurrences in a series of discrete samples (Shinozaki, 1963; Kobayashi, 1979) and Lewinsohn (1988, 1991) employed them to estimate the number of herbivore species expected on a given number of plants, using presence/absence data of the species on individual plants. By extension of the last option, they can also be used to calculate the expected number of herbivore species on a host plant at a given number of sampling sites or regions, using data on species frequencies in sites or regions, respectively.

Formally (Kobayashi, 1974, 1979; Lewinsohn, 1988, 1991) rarefaction curves are obtained by

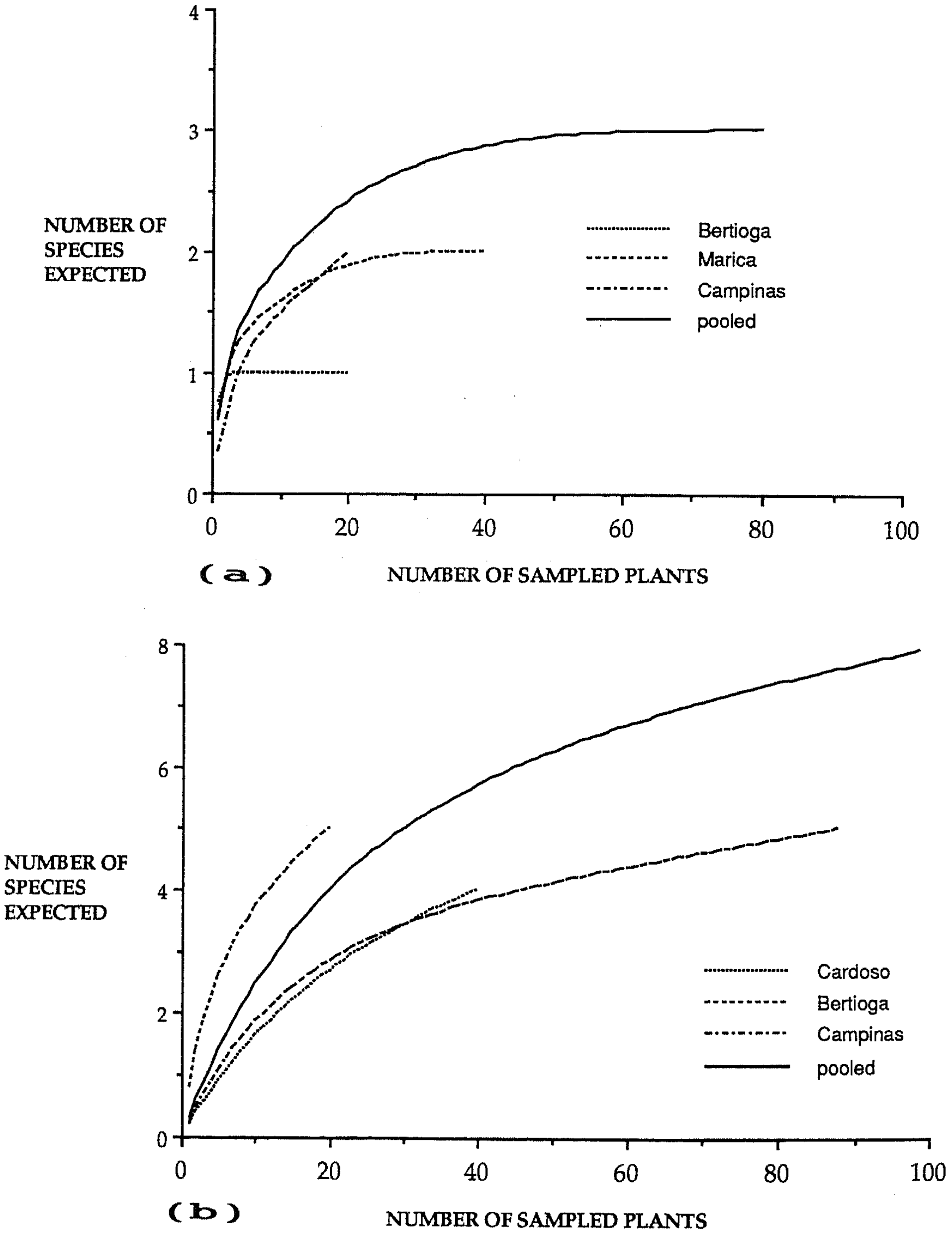
$$\bar{S}_q = S - \sum_{i=1}^{S} \binom{Q - a_i}{q} \Big/ \binom{Q}{q}$$

where $S(q)$ is the number of species expected in q sampling units (plants, sites or regions); S is the total species number recorded in the entire set of Q sampling units; a_i is the number of sampling units in which species i was recorded, that is, the absolute frequency of species i in Q; and the parentheses indicate the numbers of combinations of q units that can be chosen among, respectively, $Q - a_i$ (in a numerator) and Q (in the denominator). Thus the denominator of the fraction is equal to $Q!/q!(Q-q)!$

The rarefaction formula gives the average number of species in all possible combinations of $1, 2, \ldots q \ldots Q$ sampling units, and so it gives the number of species one would expect to have found on examining q rather than Q plants or sites. While the formula can be used to calculate $S(q)$ for any single value of q, we employ it to draw a rarefaction curve throughout the whole range of 1 to Q.

Rarefaction curves have the advantage of not being derived from a particular theoretical species-abundance distribution. They do, however, assume that species are independently distributed among whichever sampling unit (plants, localities, regions) is being considered. While this premise is questionable in several circumstances, it does not substantially affect the present use of these curves.

The performance of our sampling programme can be assessed from the shape of the rarefaction curve, although it is unsuitable for precise predictions of the number of new species to be found in a further set of samples, because extrapolation is not allowable: if we add further samples, the whole curve will shift rather than just continue beyond its present end-point. The shape of the rarefaction curve does, however, tell us whether further sampling in a locality or region is likely to reveal substantial numbers of new species, or whether it would be better to move on.



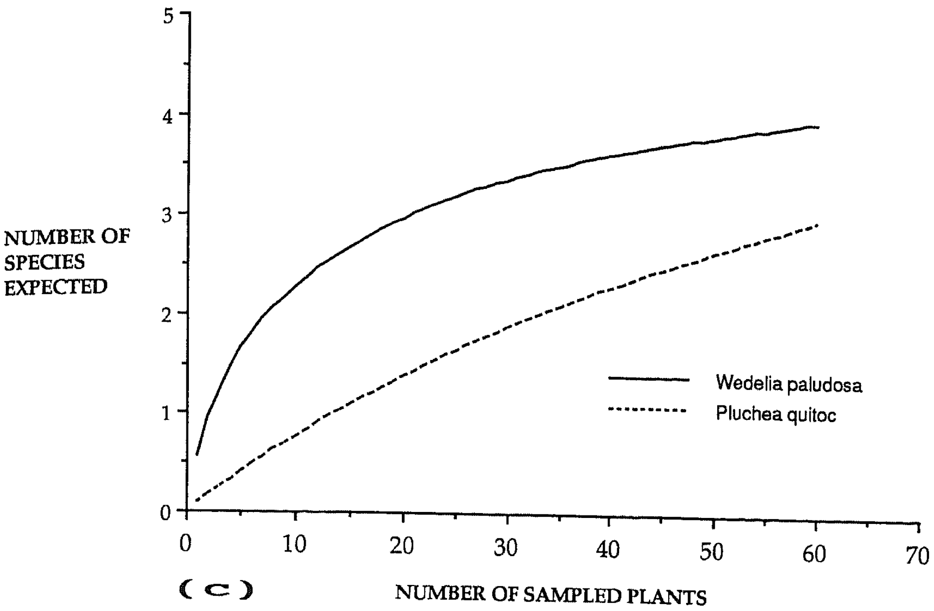


FIGURE 3. Rarefaction curves showing the number of herbivore species expected in a given number of plants for selected Asteraceae species and sites in South-east Brazil (see Lewinsohn (1988, 1991) for details). (a) Rarefaction curve for *Conyza bonariensis* at different sites; (b) rarefaction curve for *Conyza canadensis* at different sites; (c) rarefaction curve for *Wedelia paludosa* and *Pluchea quitoc* at Maricá, near Rio de Janeiro.

Model Rarefaction Curves

Based on the abundance and distribution of the herbivore species, various relationships between the number of herbivore species expected in increasing numbers of samples can be envisaged. A few simple possibilities of such rarefaction curves are depicted in Figure 2. The situation where most of the species coexist in each sampling unit is given in Figure 2(a). With regard to sites as the sampling level, this means that every site carries most of the species of the regional pool, which is the case for predominantly common and widespread herbivore species. At the other extreme (Figure 2(b)), each additional sample adds new species. This indicates predominantly rare and localized species, generating high species turnover across sampling units. Figure 2(c) represents species assemblages consisting of a mixture of few common and many rare species.

The flattening out of the curve in Figure 2(c) implies that relatively few samples are needed to discover most of the herbivore species associated with its host plant in a given area. On the other hand, Figure 2(b) suggests that additional samples will most probably lead to the discovery of further species.

CASE-STUDIES

We illustrate these ideas using data from two systems with contrasting features. The first typifies conditions of an initial survey with no previous information, whereas the second shows an extensive and in-depth study of one plant species.

Species Accumulation Across Individual Plants: Asteraceae in Brazil

Various native members of the Asteraceae were surveyed over three years in south-east Brazil, in sites ranging from coastal dunes to inland mountain ranges (Lewinsohn, 1988). This study was only concerned with species feeding endophagously on flower heads. In

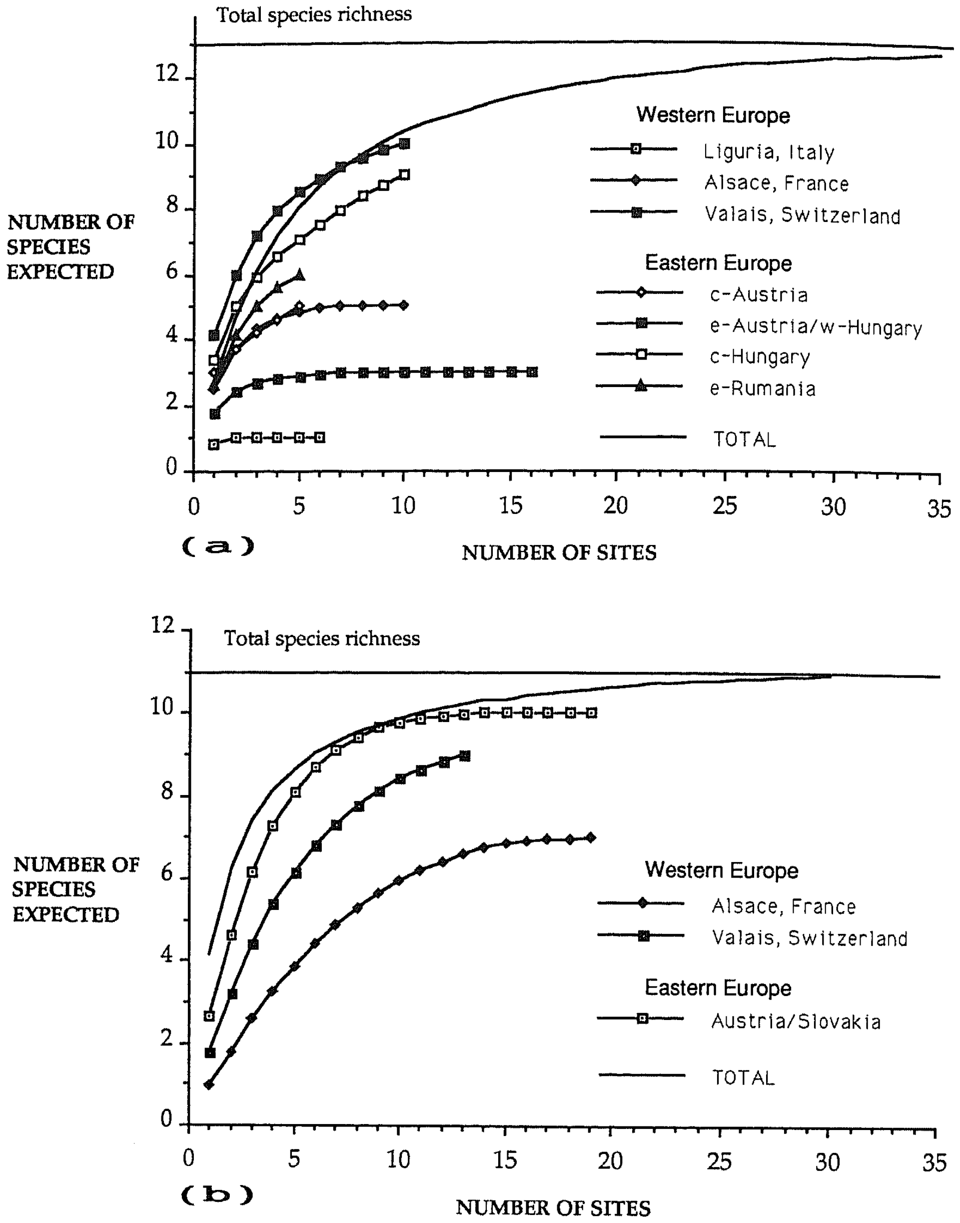


FIGURE 4. Rarefaction curves showing the number of species expected in roots and flower heads of *C. maculosa* at a given number of sites and for different regions in Europe. (a) Insects infesting the roots; (b) insects infesting the flower heads (detailed data were only available for three of the seven regions surveyed).

each locality, flower heads of 20–80 plants were collected and kept in separate containers to rear out endophages. The frequency (presence/absence) of each inset species on individual plants in each site was recorded (Lewinsohn, 1991).

Differences in the rarefaction curves for individual plants between sites and plant species are depicted in Figure 3. for a few selected weed species. The shapes of the curves vary less among sites than among plant species at different sites (Figures 3(a) and 3(b), while curves may greatly vary at a given site between plant species (Figure 3(c)) (Lewinsohn, 1991). These curves reveal, amongst other things, that fewer *Conyza bonariensis* plants need to be examined at any site to discover its local herbivores, as compared with *Conyza canadensis* plants (Figure 3(a) vs Figure 3(b)), and that the species

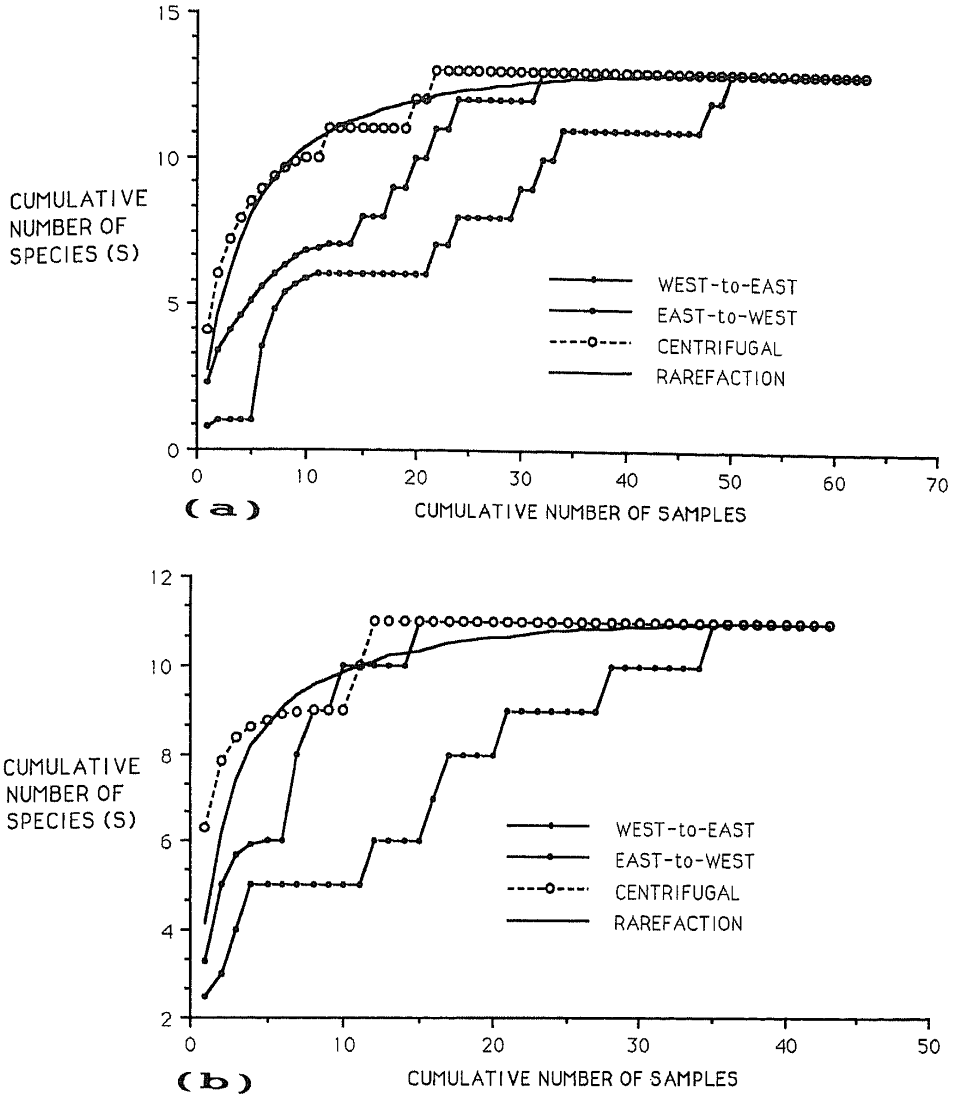


FIGURE 5. Cumulative number of insect species found on roots and flower heads of *C. maculosa* in Europe with increasing number of sites and as a function of the direction of the survey (centrifugal—starting in the plant's last glaciation refugium and extending equally to the east and west; rarefaction—expected number of species at a given number of sites, using the number of sites at which each herbivore species was found; see text for details). (a) Root insects; (b) flower head insects.

list for *Wedelia paludosa* at Maricá may be more complete than that for *Pluchea quitoc* (Figure 3(c)). This suggests that some of the plant characteristics influencing herbivore species accumulation are species attributes and hence constant across sites. Relevant plant traits may consist of both intrinsic factors, such as plant chemistry and taxonomic isolation, and extrinsic factors influencing its apparency, including longevity, architecture, patchiness and average abundance.

Species Accumulation Across Sites: *Centaurea maculosa* in Europe

Centaurea maculosa samples originate from extensive field surveys in seven regions in Europe. Samples consisted of 50–100 randomly collected roots and 100–300 flower heads per site. The number of sites per region was approximately proportional to the area occupied by the plant, and sample sites were chosen to ensure that all the habitats occupied by *C. maculosa* were investigated. Further details on sampling procedure, sites and insect species lists are given in Müller (1989) and Müller *et al.* (1989) (root insects) and in Zwölfer (1977, 1985) and Marquardt (1989) (flower head insects).

Rarefaction curves across sampling sites for *C. maculosa* are given in Figures 4 and 5, separately for different regions and root- and flower-head-infesting insects. Two different types of curves can be distinguished for the root herbivores (Figure 4(a)). The sites of the three westernmost regions—Liguria, Alsace and the Swiss Valais, where *C. maculosa* is mainly adventitious—carry relatively few species, but they occur at most of the sites. Local species assemblages in the eastern regions—Austria, Hungary and Rumania—which are closer to the glacial refugia and generally represent more stable habitats (Müller, 1989) are relatively species-rich with higher turnover of species across sites, indicating rather localized herbivore species. The rarefaction curves for the root herbivores in the more western regions suggest that most, if not all, of the herbivores have been discovered. In contrast further sampling will almost certainly reveal more species of root herbivores at the eastern sites.

Similar trends are found for flower head feeders, but they seem to consist of slightly more widespread and common species as compared to the root feeders (Figure 4(b)) (cf. Müller, 1989). Hence, fewer samples may be needed when searching for potential control organisms associated with the flower heads, as compared to below-ground herbivores.

The effect on species accumulation curves of extending the geographical area under investigation from different starting points is shown in Figure 5. Here we plot both the real species accumulation curves (analogous to Figure 1), and the calculated rarefaction curves for the entire sample. Samples were collected from western Europe to Eastern Rumania, although the presumed centre of diversification of this genus—located in southern Russia (Schroeder, 1985)—could unfortunately not be sampled. Eastern Austria/Western Hungary was a major refuge for plant species of herb-rich steppes, including *Centaurea* species, during the Pleistocene (Frenzel, 1964; 1968).

Results of both data sets show that species accumulation was fastest when starting in Eastern Austria/Western Hungary and then extending equally to east and west (centrifugal in Figures 5(a) and 5(b)). In this case, the first 23 root samples and 12 flower head samples, respectively, revealed all the herbivore species discovered during the entire survey. This confirms the suggestion that, when possible, surveys for weed biological control agents should start in biogeographic refugia or centres of endemism. Starting in western Europe, where the plant is adventitious, yielded the slowest species accumulation rate.

GUIDELINES FOR A SURVEY PROTOCOL USING RAREFACTION CURVES

We can now use these examples to suggest guidelines for the way in which rarefaction curves could be used to increase survey efficiency. Assume, first, that work is taking place within one geographic region, and the problem is to decide how many plants to look at in one collection site, and when to move on to another site.

First, we advocate random coverage of approximately 10 sites within the region, involving the examination of, say, 50–100 plants at each site, much as advocated in the guidelines of Schroeder (1983) and Schroeder & Goeden (1986), and in widely used general protocols. Because different species of insects have different phenologies, each site should, ideally, be sampled more than once during the growing season. This is especially important when sampling plant species that occur in environments differing widely in seasonality. From such a data set individual and pooled rarefaction curves can be generated as we have shown. Such curves will indicate (a) which sites possibly contain more undiscovered herbivore species, and (b) whether sampling of new sites is more likely to reveal further herbivores. When the rarefaction curves have levelled off, it will generally be more cost-effective to move to another site, or to a new region. The advantages of the rarefaction curve are made particularly clear by data in Figure 5. From the simple species accumulation curve ("collector's curve") for samples ordered from west to east, for example, it is difficult to decide whether collections are nearing completion. However, in combination with the rarefaction curve, it is clear that few if any insect species remain to be discovered in this region.

Sampling in several geographical regions basically reproduces, at a further level, the above procedures. However, at this stage financial and political considerations may be additional important constraints. Finally, as previously has been argued (Wapshere, 1974, 1981;Schroeder & Goeden, 1986) and as Figure 5 makes plain, surveys in the host plant's Pleistocene refugia or in centres of endemism for the taxon may maximize the rate of discovery of potential control agents. However, even here, survey efficiency may be improved within and between sites, by constructing rarefaction curves as field-work progresses.

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REFERENCES

COMPTON, S.G., LAWTON, J.H. & RASHBROOK, V.K. (1989) Regional diversity, local community structure and vacant niches: the herbivorous arthropods of bracken in South Africa. *Ecological Entomology* **14,** 365–373.

FRENZEL, B. (1964) Über die offene Vegetation der letzten Eiszeit am Ostrande der Alpen. *Verhandlungen der Zoologischen und Botanischen Gesellschaft Wien* **103/104,** 110–143.

FRENZEL, B. (1968) The Pleistocene vegetation of Northern Eurasia. *Science* **161,** 637–649.

HOPKINS, B. (1955) The species–area relation of plant communities. *Journal of Ecology* **43,** 409–426.

HURLBERT, S.H. (1971) The nonconcept of species diversity: a critique and alternative parameters. *Ecology* **52,** 577–586.

KOBAYASHI, S. (1974) The species–area relation. I. A model for discrete sampling. *Researches on Population Ecology* **15,** 223–237.

KOBAYASHI, S. (1979) Species–area curves, in *Statistical Distributions in Ecological Work* (ORD, J.K., PATIL, G.P. & TAILLIE, C., Eds.) International Cooperative Publishing House, Fairland, MD, pp. 349–368.

KREBS, C.J. (1989) *Ecological Methodology.* Harper and Row, New York.

LAWTON, J.H. (1988) Biological control of bracken in Britain: constraints and opportunities. *Philosophical Transactions of the Royal Society of London B* **318,** 335–355.

LAWTON, J.H. (1990) The U.K. biological control programme for bracken, in *Alternatives to the Chemical Control of Weeds* (BASSETT, C., WHITEHOUSE, L. & ZABKIEWICZ, J.A., Eds.) *Proceedings of an International Conference, Rotorua, New Zealand, July 1989.* Ministry of Forestry, *FRI Bulletin* **155,** 34–39.

LEWINSOHN, T.M. (1988) Composição e tamanho de faunas associadas a capitulos de Compositas. *DSc Thesis,* Unicamp, Campinas, Brazil.

LEWISOHN, T.M. (1991) Insects in flower heads of Asteraceae in Southest Brazil: a tropical case study on species richness, in *Plant–Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions* (PRICE, P.W., LEWINSOHN, T.M., FERNANDEZ, G.W. & BENSON, W.W., Eds.) John Wiley, New York, pp. 525–559.

MAGURRAN, A.E. (1988) *Ecological diversity and its measurements.* Croom Helm, London.

MARQUARDT, K. (1989) Biologie, Ökologie und Wirtskreis von *Chaetorellia acrolophi* White and *Terellia virens* (Loew) (Diptera: Tephritidae), zwei potentielle Kandidaten für die biologische Bekämpfung von *Centaurea maculosa* Lam. und *C. diffusa* Lam. (Asteraceae) in Nordamerika. *PhD Thesis,* University of Kiel, Germany.

MAY, R.M. (1975) Patterns of species abundance and diversity, in *Ecology and Evolution of Communities* (COY, M.L. & DIAMOND, J.M., Eds.) Belknap, Cambridge, MA, pp. 81–120.

MCGUINNESS, K.A. (1984) Equations and explanations in the study of species–area curves. *Biological Reviews* **59,** 423–440.

MÜLLER, H. (1989) Structural analysis of the phytophagous insect guilds associated with the roots of *Centaurea maculosa* Lam., *C. diffusa* Lam. and *C. vallesiaca* Jordan in Europe: 1. Field observations. *Oecologia* **78,** 41–52.

MÜLLER, H. (1990) An experimental and phytocentric approach for selecting effective biological control agents: Insects on spotted and diffuse knapweed, *Centaurea maculosa* Lam, and *C. diffusa Lam. (Compositae)*, in

Proceedings of VII International Symposium on Biological Control of Weeds, Rome, 1988 (Delfosse, E.S., Ed.) pp. 181–190.

Müller, H., Stinson, C.S.A., Marquardt, K. & Schroeder, D. (1989) The entomofaunas of roots of *Centaurea maculosa* Lam., *C. diffusa* Lam. and *C. vallesiaca* Jordan in Europe: niche separation in space and time. *Journal of Applied Entomology* **107,** 83–95.

Sanders, H.L. (1968) Marine benthic diversity: a comparative study. *American Naturalist* **102,** 243–282.

Schroeder, D. (1983) Biological control of weeds, in *Recent Advances in Weed Research* (Fletcher, W., Ed.) Commonwealth Agricultural Bureau, Slough, pp. 41–78.

Schroeder, D. (1985) The search for effective biological control agents in Europe: 1. Diffuse and spotted knapweed, in *Proceedings of VI International Symposium on Biological Control of Weeds, Vancouver*, (Delfosse, E., Ed.) Agriculture Canada, Ottawa, pp. 103–119.

Schroeder, D. & Goeden, R.D. (1986) The search for arthropod natural enemies of introduced weeds for biological control: in theory and practice. *Biocontrol News and Information* **7,** 147–155.

Shinozaki, K. (1963) Note on the species–area curve, in *Proceedings of the 10th Annual Meeting of the Ecological Society of Japan, Tokyo*, pp. 1–5.

Southwood, T.R.E. (1978) *Ecological Methods, with Particular Reference to the Study of Insect Populations*, Chapman and Hall, London and New York, 2nd edn.

Wapshere, A.J. (1974) Host specificity of phytophagous organisms and the evolutionary centres of plant genera and subgenera. *Entomophaga* **19,** 301–309.

Wapshere, A.J. (1981) Recent thoughts on exploration and discovery for biological control of weeds, in *Proceedings of V International Symposium Biological Control of Weeds, Brisbane, Australia, 1980* (Delposse, E.S., Ed.) CSIRO, Melbourne, pp. 75–79.

Zwölfer, H. (1977) An analysis of the insect complex associated with the head of European *Centaurea maculosa* populations, in *Proceedings of the Knapweed Symposium, Kamloops, Canada*, pp. 139–163.

Zwölfer, H. (1985) Insects and thistle heads: resource utilization and guild structure, in *Proceedings of VI International Symposium Biological Control of Weeds, Vancouver*, (Delfosse, E.S., Ed.) Agriculture Canada, Ottawa, pp. 407–416.