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Comments on the dynamics of insect population assemblages and sampling plans for aphids in commercial alpine yarrow fields

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Abstract

Achillea collina Becker ex Rchb. (yarrow) is a medicinal plant grown for commercial purposes in southern Switzerland and northern Italy. The economically most relevant population assemblage is organized around the phloem feeding aphids *Macrosiphoniella millefolii* (De Geer), *Aphis spiraecola* Patch and *Coloradoa achilleae* Hille Ris Lambers. The study is a contribution to explaining the dynamics of this assemblage in two Alpine fields, on the basis of multitrophic population interactions. The method consisted in stratifying the field and taking random samples of yarrow stems and leaves, aphid numbers, and numbers of adult coccinellids and mummified aphids throughout the growing period. There were no significant differences in aphid densities between the strata ($\alpha = 0.05$; $F = 1.561$; $P = 0.138$). Once the photoperiod reaches 14 h, a time window opens for stem elongation and reproductive development that creates suitable conditions for the aphids. There are indications for an influence of the plant and a possibly limited effect of natural enemies on aphid infestations. The coefficients b (1.920, 1.917) and a (3.911, 14.539) of Taylor's power law indicate aggregated distributions of *M. millefolii* and *A. spiraecola*. To obtain a reliable density estimate, 100 or 400 plants should be sampled for *M. millefolii* and *A. spiraecola*, respectively. An enumerative sequential sampling plan appears to be more efficient than the enumerative fixed sample size sampling plan.

Key words: *Achillea collina*, *Macrosiphoniella millefolii*, *Aphis spiraecola*, *Coloradoa achilleae*, temporal dynamics, spatial distribution.

Introduction

An ecosystem is a unit which supports life and includes all biological and non-biological variables in that unit (Jørgensen, 2002). According to the demographic approach to ecosystem study and management, interacting populations that operate at different trophic levels are constituent ecosystem elements linked through flows of biomass, energy and nutrients (Gutierrez, 1996). The ecosystemic view to the dynamics and regulation of insect populations considers a population and the functional aspects of its environment as one subsystem of a hierarchically structured ecosystem (Huffaker *et al.*, 1999). To support growth, maintenance and reproduction, the different populations acquire resources of different quality and acceptability as available (Schowalter, 2006). Resource quality mainly depends on resource requirements, variation in food quality and chemical defence systems; resource acceptability is determined by feeding preference, while the resource availability depends on the abundance, distribution, and appearance of acceptable resources. The acquisition of resources and the subsequent allocation to growth, maintenance and reproduction profoundly affect the strength of the interactions and the spatio-temporal dynamics of populations (Gutierrez, 1996; Schowalter, 2006).

In general, the analysis of the structure, the function and the evolution of ecosystems is a challenging task, mainly because of the high number of constituent populations and possible interactions (Jørgensen, 2002). According to the strength of population interactions, ecologists distinguish between different subsystems or groupings such as communities and population assemblages as referred to in this paper. The term is preferred over community because it is less restrictive and not referring to a given taxonomic group taken with a given sampling technique (Garcia *et al.*, 2003). The demographic approach to ecosystem study and management focus on economically relevant assemblages of interacting plants, plant subunits (fruits, leaves, stems, roots), herbivore populations, and their natural enemies (Gutierrez, 1996; Gutierrez and Baumgärtner, 2007). At the field level, the study and management of population assemblages, organized around economically relevant herbivore populations, relies on knowledge of population densities changing in time and space. Reliable estimates of population densities can be obtained through the development of adequate sampling plans (Cochran, 1963; Pedigo and Buntin, 1993).

Achillea collina Becker ex Rchb. (Asteraceae) is a medicinal plant belonging to the *Achillea millefolium* L. aggregate (yarrow), a polymorphic aggregate consisting of about 20 species that differ in ploidy level, morphology and chemical composition (Giorgi *et al.*, 2005). *A. collina* is cultivated in European alpine areas and commonly used as aqueous and alcoholic extracts for their digestive, antiphlogistic, spasmolytic, stomachic, carminative, estrogenic and antioxidant properties

(Benedek *et al.*, 2007; Giorgi *et al.*, 2009). This plant species is tolerant to low temperatures and drought, and survives in temperate climates of the boreal hemisphere up to 1800 m a.s.l. Although yarrow prefers environments with high radiation, it also develops at locations exposed to as little as 4-5 hours of sunshine (Cernaj and Helemikova, 1991). The soil doesn't have to be rich but should be well drained to avoid water logging detrimental to yarrow roots (Cernaj and Helemikova, 1991). The influence of habitat features on growth, development, biomass yield and phenolics content in *A. collina* as well as on the essential oil production in *Achillea filipendulina* L. was elucidated by Giorgi *et al.* (2010a) and Mosayebi *et al.* (2008), respectively.

Commercial *A. collina* fields in the alpine regions of southeastern Switzerland and northern Italy are inhabited by many insect populations including aphids of possible economic importance. The purpose of this study is to identify population assemblages organized around phytophagous insect populations with potential impact on *A. collina* growth, development and yield. The study on the spatio-temporal distribution of the population assemblage organized around phloem feeding aphids seeks to improve the knowledge on the economic importance of aphids and on factors influencing the dynamics of their populations. In addition, the study aims at the development of sampling plans to efficiently estimate aphid population densities with predefined levels of reliability.

Materials and methods

Experimental fields and environmental conditions

The study was conducted between 2007 and 2009 in two yarrow fields located at Poschiavo (1140 m a.s.l., Canton of the Grisons, Switzerland) and Dazio (568 m a.s.l., Province of Sondrio, Italy). At both locations, plants of *A. collina* cv. "SPAK" (provided by Valplantos Bio SAILLON - CH), were planted at 30 cm intervals in rows 40 cm apart, resulting in a plant density of 6.5 plants/m^2 and grown under a plastic mulch suppressing weeds. To obtain the maximum amount of secondary metabolites, the crop is harvested in the morning as soon as 70% of the plants are flowering.

The field at Poschiavo was established in 2002, and yarrow was harvested once per year in July until spring of 2009. The Dazio field, established in 2006, was never harvested during our study and consequently, had higher and more vigorous plants than at Poschiavo. The Poschiavo field was surrounded by fields of other medicinal plants, while the Dazio field was located within a poorly managed area with spontaneous alpine vegetation, shrubs and trees.

The daily mean temperatures and the daily precipitations for Robbia-Poschiavo and Morbegno-Dazio were obtained from MeteoSwiss and ARPA, respectively. The daily photoperiods for Poschiavo (46º19'42") and Dazio (46º09'45") were calculated with Glarner's (2010) algorithm.

Plant sampling

To take into account the growth patterns of yarrow, we modified a simple sampling system that represents vegetative and reproductive growth under photoperiodic influence (Cline and Agatep, 1970). Accordingly, stem elongation and reproductive development are initiated by a photophase exceeding 14 h. Plant samples were taken in 2008 in Poschiavo and in 2009 in Dazio. The basal part of the plant consists of basal leaves and stems with associated leaves. At the beginning of the growing season, the number of basal leaves and the number of stems were counted on 5 randomly selected plants. The number of plants counted was reduced to 3 after the beginning of stem elongation. The mean number of basal leaves and stems per plant as well as the sampling dates are given in figure 1.

Insect sampling

A sampling program delineates the sampling universe, the sampling technique, the sample unit size, the number of sample units, the patterns of collecting sample units, and the timing of samples (Pedigo and Buntin, 1993). The yarrow fields in Poschiavo and Dazio were subdivided into 9 and 6 rectangular plots or strata of equal dimensions. In each stratum, the beating tray technique was applied to 3 randomly selected plants, i.e. sample units. The sampling dates are given in table 1. For each plant, the insects falling into a plastic container (Ø 12.5 cm; h 11 cm) were put into vials (Ø 6.3 cm, h 8 cm) filled with 70% alcohol and transported to the laboratory for species identification and counts of mummified aphids. In the case of aphids, mites and thrips, we used the Pedigo counting mask for obtaining densities (Pedigo and Buntin, 1993). Based on both information on herbivore abundance and on entomological knowledge on the strength of the interactions determined by the acquisition of resources and their allocation (Schowalter, 2006), population assemblages of possible economical relevance were constructed and inserted in table 1.

Spatial distribution of aphids

The spatial distribution was analysed with the samples taken in Poschiavo (2007 and 2008) and Dazio (2008). To stabilize the variance, the data on the most abundant aphids *Macrosiphoniella millefolii* (De Geer) and *Aphis spiraecola* Patch were square root transformed (Zar, 1974). This was necessary since the homogeneity of the variance was rejected by Levene's (1960) test ($\alpha = 0.05$; $F = 4.509$; $P < 0.001$). The transformed counts were subjected to an analysis of variance for evaluating the differences between the strata. Taylor's (1961) power law was used to express the relationship between the mean (m) and the variance (s^2)

$$s^2 = am^b \qquad [1]$$

where a and b are defined as sampling factor and species-specific distribution parameter, respectively. Among many others, Knapp *et al.* (2003) found it convenient to take the natural logarithm and estimate the parameters ln (a) and (b) via least square regression techniques.

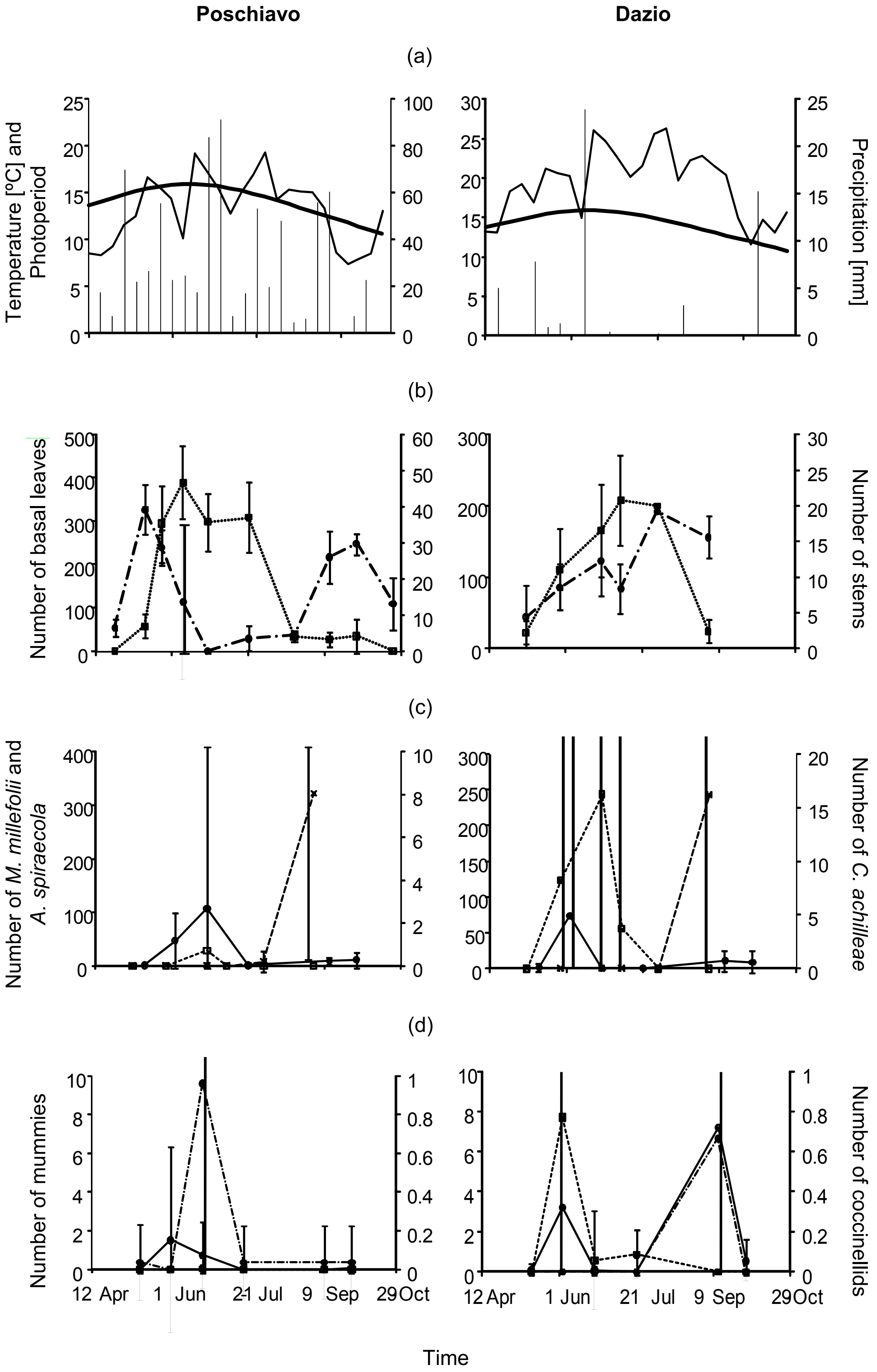

Figure 1. Environmental conditions (a): weekly mean temperatures (continuous line), precipitations (bars) and photoperiod (bold line); plant sub-populations (b): number of basal leaves per plant (dash-dotted line) and stems per plant (dotted line); number of phytophagous insects per plant (c): *M. millefolii* (continuous line), *A. spiraecola* (dashed line) and *C. achilleae* (long dashed line); number of natural enemies per plant (d): adult *C. septempunctata* (long dash-dotted line), *M. millefolii* mummies (continuous line) and *A. spiraecola* mummies (dashed line), in two commercial alpine yarrow fields. Points represent the mean observed data per plant, and the bars are the calculated standard errors; error bars have been truncated for a better visualization of the graphs (plant samples were taken in 2008 in Poschiavo and in 2009 in Dazio; insect samples were taken in 2008 at both location).

Table 1. List of the most abundant insect species recorded in 2007 and 2008 in the Poschiavo and Dazio commercial yarrow fields (Calendar dates refer to the first occurrences).

Species	Year of observation			
	2007		2008	
	Poschiavo	Dazio	Poschiavo	Dazio
Macrosiphoniella millefolii (DeGeer) (Hemiptera Aphididae)	April 28th	-	May 14th	May 14th
Aphis spiraecola Patch (Hemiptera Aphididae)	April 28th	-	June 3rd	June 3rd
Colorodoa achilleae Hille Ris Lambers (Hemiptera Aphididae)	September 5th	-	September 12th	September 30th
Galeruca tanaceti (L.) (Coleoptera Chrysomelidae)	April 24th	May 9th	June 24th	June 3rd
Chrysolina marginata (L.) (Coleoptera Chrysomelidae)	May 9th	-	-	-
Cassida sp. (L.) (Coleoptera Chrysomelidae)	-	May 25th	-	June 3rd
Parasitoids: mummified aphids (Hymenoptera Braconidae)	April 24th	May 9th	May 14th	June 3rd
Predators: larvae and pupae (Diptera Syrphidae)	May 25th	May 9th	June 24th	September 12th
Predators: larvae and pupae (*Coccinella septempunctata* L.)	May 25th	May 9th	May 14th	September 12th

Enumerative sampling plan

The smallest number n of sampling units (plants) to be taken for satisfying predefined reliability criteria for the estimate is referred to as optimum sample size (Karandinos, 1976). For the definition of reliability, we rely on formal probabilistic statements with the ratio D of the standard error to the mean (Karandinos, 1976). The use of the sample statistics s^2 and m and the substitution of the variance by equation [1] yields

$$n = \left(\frac{z_{\alpha/2}}{D} \right)^2 a m^{(b-2)} \qquad [2]$$

where $z_{\alpha/2}$ is the standard normal deviate. For the purpose of this work, $D = 0.3$ and $z_{\alpha/2} = 1.65$ for $P = 0.1$ were considered as satisfactory.

Sequential enumerative sampling plan

According to this plan, sampling begins with 12 sampling units (Pickel *et al.*, 1983; Bianchi *et al.*, 1989) and continues until the cumulative number of individuals has reached or exceeded a stop-line. The modified equation (29) in Hutchison (1993) was used to calculate the stop line for the most abundant aphids *M. millefolii* and *A. spiraecola*

$$\ln(T_n) = \left(\frac{\ln\left(\frac{D^2}{z^2_{\alpha/2}} a \right)}{(b-2)} \right) + [(b-1)/(b-2)]\ln(n) \qquad [3]$$

where T_n is the cumulative number of individuals, D is the ratio of the standard error to the mean, n is the number of sampling units, and a and b are Taylor's (1961) parameters derived for both species from equation [1]. Again for the purpose of this work, $D = 0.3$ and $z_{\alpha/2} = 1.65$ for $P = 0.1$ were considered as satisfactory. The use of $D = 0.13$ for *M. millefolii* and $D = 0.06$ for *A. spiraecola* takes into account a relatively high level of reliability and allows sampling in a similar range of plant samples (n) for both species.

Results

Definition and importance of population assemblages

Table 1 reports the most abundant phytophagous insect populations and their natural enemies found. Accordingly, there are two main assemblages organized around either three species of phloem-feeding aphids (Aphididae) or three species of leaf-eating leaf beetles (Chrysomelidae). A third and a fourth assemblage of limited economic importance not reported here is organized around thrips (Thripidae) and mirids (Miridae), respectively. This study focuses on aphid populations. Their phloem feeding habit and their densities (figure 1b) indicate that they are at the centre of the economically most relevant population assemblage. Table 1 also lists the natural enemies of aphids that were coccinellids, syrphids and microhymenopteran parasitoids.

Environmental conditions

Figure 1a shows the weekly mean temperatures, precipitations and the photoperiod recorded in 2008 at the Poschiavo-Robbia (800 m a.s.l.) and Morbegno-Dazio (300 m a.s.l.) weather stations. After the beginning of March, the daily mean temperatures at both locations are consistently above the thresholds of 2.5 ºC allowing plant development under presumably favourable levels of radiation. In Morbegno-Dazio, the rainfall was smaller and less frequent than in Poschiavo. Because of the fitness of *A. collina* to dry conditions, even the precipitations in Dazio might have been sufficient to prevent a yield-limiting water stress. Figure 1b shows that the mean number of stems per plant begins to increase at the beginning of May at both sites, i.e. after April 27 and April 29 when the 14 hours day length is reached in Poschiavo and Dazio, respectively. Apparently, the SPAK cultivar studied here falls into the group of cultivars that initiate reproductive growth at a 14 h photoperiod.

Patterns of plant growth

Figure 1b reports the number of stems and basal leaves per plant at the Poschiavo (2008) and Dazio (2009) sites. The number of basal leaves increases until the photoperiod allows the growth of reproductive units (stems and their leaves) and decreases thereafter. Interestingly, the change in the number of reproductive units during the summer is similar at both locations, although the Poschiavo field was cut for commercial purpose at the beginning of July while no harvest occurred at Dazio. Apparently, the reserves available to the plants in Poschiavo and the environmental conditions allow the crop to build-up the same number of stems that were present before the harvest. The number of basal leaves increases at the beginning of September, presumably because the decreasing photoperiod terminates reproductive growth and available photosynthates permit again vegetative growth.

With few exceptions, the standard errors of counts are relatively small despite of the low number of 3-5 plant examined. This indicates a small variability among the plants in the two commercial yarrow fields. A high level of reliability in estimates on basal leaf numbers and stems can be reached with relatively small sample sizes of less than 10 plants.

Insect infestation patterns

Figure 1c depicts the fluctuations of *M. millefolii*, *A. spiraecola* and *Coloradoa achilleae* Hille Ris Lambers in the Poschiavo (2008) and Dazio (2008) yarrow fields. The analysis of variance conducted for the counts of the first two species lead to the acceptation of the null hypothesis according to that there were no significant differences between the strata ($\alpha = 0.05$; $F = 1.561$; $P = 0.138$). Hence, the sampling universe is considered as homogeneous and the samples are treated as simple random samples in the development of sampling plans.

Figure 1c shows that the density estimates for all aphids are associated with high standard errors. In fact, the emphasis given to densities prevented us from a complete representation of the bars in the picture. The high variability between the counts within a sample will be discussed in the subsequent section. Of interest in this section is the temporal occurrence of insects and the difference in insect presence between the sites. Accordingly, *C. achilleae* was present at both locations, during the absence of the other two species, in the late season only. *M. millefolii* and *A. spiraecola*, however, occur from May to August. At the Poschiavo site, *M. millefolii* was more abundant than *A. spiraecola*. This was already observed in a preliminary survey conducted in the previous year (2006) and not included into this investigation.

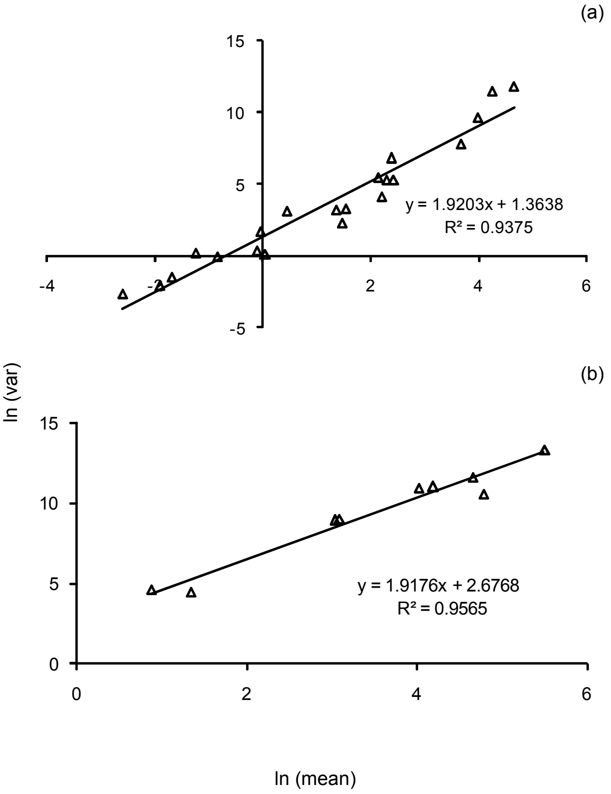

Figure 2. Taylor's (1961) power law representing the relationship between the natural logarithm of the variance ln (var) and the logarithm of the mean ln (mean) for *M. millefolii* (a) and *A. spiraecola* (b) densities per plant in commercial alpine yarrow fields.

At the time of the infestation by these two aphid species, we observed high number of mummified aphids and coccinellid predators (figure 1d). The number of mummies was higher in Dazio than in Poschiavo. Since we only occasionally observed Syrphidae in the yarrow fields, aphid predation is mainly attributed to *Coccinella septempunctata* L.

Spatial distributions

Figure 2 shows the relationship between the logarithm of the variance and the logarithm of the mean for *M. millefolii* and *A. spiraecola*. The high correlation coefficients and the visual examination of figure 2 show that equation [1] satisfactorily describes the relationships. The values for b are 1.9203 and 1.9176 and for a are exp(1.3638) = 3.911 and exp(2.6768) = 14.539, respectively. This indicates that the two species are highly aggregated.

Enumerative fixed sample size sampling plan

Figure 3 shows the optimum sample size n in relation to the mean densities of *M. millefolii* and *A. spiraecola* according to equation [2]. The definition of the reliability of the estimates is based on formal probabilistic statements with the ratio D of the standard error to the mean. The here selected values for the standard normal deviate $z_{\alpha/2} = 1.65$ for $P = 0.1$ and $D = 0.3$ reflect the acceptance of a relatively low level of reliability. If the sampling program requires a higher level of reliability, the parameters can easily be changed and figure 3 can be adapted accordingly. Figure 3 shows that the sample size, at medium and high densities, should be about 100 plants for *M. millefolii* and about 400 plants for *A. spiraecola*.

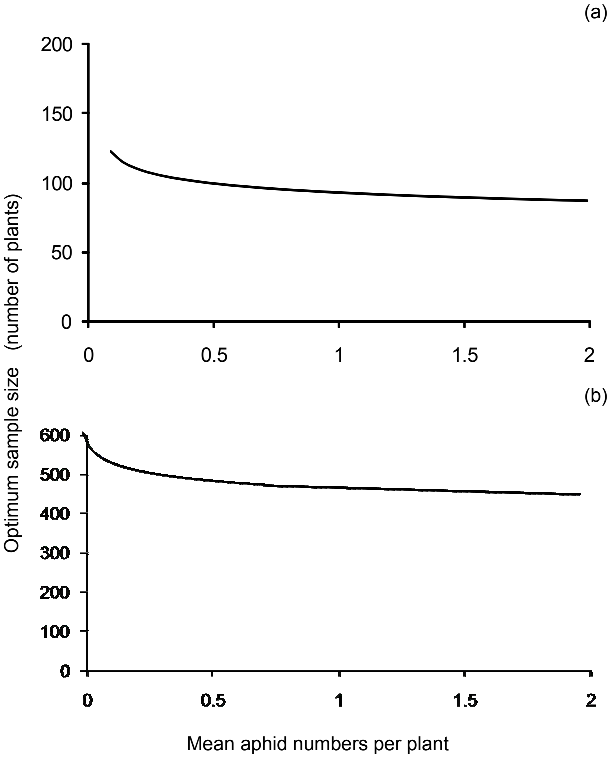

Figure 3. The optimum sample size in relation to mean densities of *M. millefolii* (a) and *A. spiraecola* (b) per plant in commercial alpine yarrow field. The reliability is based on formal probabilistic statements ($z_{\alpha/2}$ = 1.65 for P = 0.1, D = 0.3 as the ratio of the standard error to the mean).

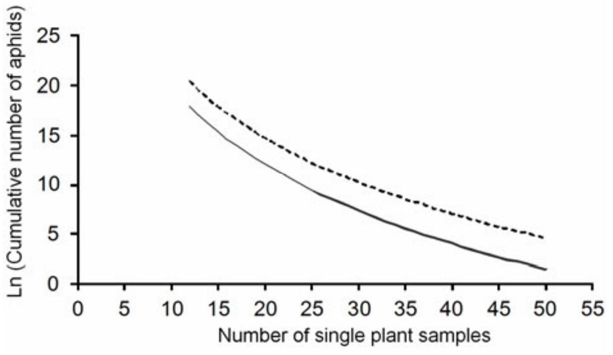

Figure 4. The stop lines in the enumerative sequential sampling plan for *M. millefolii* (continuous line) and *A. spiraecola* (dashed line) in commercial alpine yarrow field. The reliability is based on formal probabilistic statements ($z_{\alpha/2}$ = 1.65 for P = 0.1, D = 0.13 and 0.06, as the ratio of the standard error to the mean, for *M. millefolii* and *A. spiraecola*, respectively).

Enumerative sequential sampling plan

Figure 4 shows the stop lines obtained through equation [3] in the enumerative sequential sampling plan for *M. millefolii* and *A. spiraecola.* Accordingly, the sampling procedure should start with an initial sample size of 11 plants. The aphids found in this initial sample should be summed up. Additional plants are selected and the aphids found should be added to the number of aphids already found. This activity should be continued until the accumulated number of aphids reaches or exceeds the stop line.

Discussion

The competition for photosynthate modulated by photoperiod may be responsible for the growth pattern of basal leaves and stems depicted in figure 1b. In fact, the cessation of basal leaf formation coincides with the photoperiod-controlled growth of stems and the formation of reproductive structures. In demographic ecosystem analyses, competition for photosynthate among plant units and priority given to some units in the photosynthate allocation have been identified as a key processes in crop yield formation (e.g. Gutierrez, 1996; Gutierrez and Baumgärtner, 2007). The observations made at Poschiavo (figure 1b) indicate that both the harvesting and the subsequent restricted stem growth permit re-growth of basal leaves. The growth of basal leaves is also possible when decreased daylenght terminates reproductive development. This response is less clear at Dazio where the crop was not harvested during the period under observation (figure 1b). At Dazio, we occasionally measured the weight of rhizomes (not reported here), which are storage organs for reserves, and observed that the fresh weight decreased during the vegetative and reproductive periods. The cessation of basal leaf growth is presumably due to low temperatures in fall and to the transfer of carbohydrates from leaves to rhizomes.

The studies at the Poschiavo and Dazio sites indicate that the population assemblage organized around aphids is important from the economic standpoint. However, the available information does not allow to generalize the findings and the relative importance of the assemblages defined above may change at other sites. The plant growth pattern has a profound influence on phloem feeding aphids (Gutierrez and Baumgärtner, 1984; Holst and Ruggle, 1997). This is indicated by the infestations of *M. millefolii* and *A. spiraecola* that coincide with early stem growth and of *C. achilleae* that occur during reduced stem growth in the late season (figure 1a, 1b). However, the earliness of the infestation may have been influenced to some extent by the plastic mulch, i.e. a black plastic promoting crop growth and earliness by increasing soil temperature (Kumar and Lal, 2012). Yellow plastic sheet traps recorded an early presence of *Myzus persicae* Sulzer on potatoes (Jan *et al*., 2002). The influence of the temperatures on the periods of presence of parthenogenetic morphs appears be limited but may be decisive for the expected decrease of the aphids in the late season (figure 1c). The influence of the temperature on aphid densities, however, may be substantial. Namely, the preliminary simulation study carried out by Gama *et al*. (2010) showed that the temperature conditions at the alpine sites are not favourable for *A. spiraecola.* This is also supported by figure 1c where *A. spiraecola*, in contrast to *M. millefolii*, reaches higher densities at Dazio with higher temperatures than Poschiavo. The life tables published by Morlacchi *et al.* (2011) further support the observation that *M. millefolii* is better adapted to alpine environments than *A. spireacola.* The competition for carbohydrates among plant parts may have restricted food uptake in relation to food demand by the aphids and influenced the formation of winged morphs, not recorded here, whose migration may have contributed to the here observed decrease in aphid densities (see Gutierrez, 1996, Holst and Ruggle, 1997). Previous studies demonstrated that the defence system of yarrow can be activated by phloem feeding *M. persicae* (Giorgi *et al*., 2010b; Nanayakkarawasam Masachchige *et al*., 2011; Giorgi *et al*., 2012). The activation resulted in decreased intrinsic rates of increase for this species and may complement the aforementioned temperature influence on aphid dynamics. In laboratory experiments, *M. persicae* reduced the growth of yarrow and increased secondary metabolites (Giorgi *et al*., 2010b; Nanayakkarawasam Masachchige *et al*., 2011; Giorgi *et al.,* 2012). However, their synthesis may come at a cost to the plant. Assuming that *M. millefolii* and *A. spiraecola* have similar effects on growth and biochemistry of yarrow and taking into account their relatively high densities (figure 1), these observations confirm the supposition that aphids are economically relevant components of the Alpine yarrow ecosystem.

In general, herbivorous insects are not randomly dispersed among their host plants but rather they attack some species or individuals preferentially over others (Downing, 1986). This preference could be the result of several factors, such as the dispersal abilities of the insect, environmental variation, the heterogeneous effects of parasites and predators, or variation between host plants in their nutritional quality, susceptibility or defensive phenotypes (Strauss, 1990). Thus, the here observed heterogeneous distribution of the aphids on yarrow host plants could be a function of the varying degrees of susceptibility in the host plant population (Wardhaugh and Didham, 2006).

Figure 1d suggests that the number of mummies and predators appear to respond with a time delay to increasing and decreasing *M. millefolii* and *A. spiraecola* populations. The delay and the low densities of mummies and predators (figure 1d) may indicate a limited impact of natural enemies. This is not in contrast to a recent literature review that concludes that natural enemy activity is important in some but not all studies on aphid population dynamics (Brown and Mathews, 2008). However, the information restricted to time delays and densities is insufficient for conclusively explaining the impact of predators and parasitoids under the influence of the here observed aggregated distributions. Namely, a satisfactory explanation of natural enemy impact should also take into account the non-random search patterns of predators (Waage, 1979; Ryoo, 1996) and the implications of aphid attendance by ants that are attracted by honey dew producing *A. spiraecola* colonies and defend them from natural enemy attacks (see Schowalter, 2006). In the case of *M. millefolii,* the evaluation of natural enemies should also consider the mimicking of the colour of flower structures which may provide some protection from natural enemy attack (Hille Ris Lambers, 1938). The explanation of biological control by *C. septempunctata* and unidentified microhymenopteran parasites is further complicated by the influence of host plant species acting through *M. persicae* and *M. millefolii* on *C. septempunctata*. As a result, the performance of *C. septempunctata* may depend on the presence of aphid species (Ali and Rizvi, 2007).

Miller (2008) stated that it is now widely accepted that herbivore dynamics can be influenced by both bottom-up and top-down forces, and their relative importance can vary spatially and temporally. There are indications for an influence of the plant and a possibly limited effect of natural enemies on aphid infestations. These indications, however, are too vague as to allow a qualification and quantification of forces in a bottom-up *versus* top-down context. Experimental studies and the development of mechanistic models hold the promise to improve the insight into dynamics of the yarrow – aphid – natural enemy population assemblage and to allow final conclusions regarding the effect of spatially and temporally varying bottom-up and top-down forces (Gutierrez, 1996; Huffaker *et al.*, 1999; Jørgensen, 2002; Gutierrez and Baumgärtner, 2007). A mechanistic model may also be able to represent the acquisition of resources and the subsequent allocation to growth, maintenance and reproduction that profoundly affect the strength of the interactions and the spatio-temporal dynamics of populations (Gutierrez, 1996; Schowalter, 2006).

The high sample sizes are due to the high values obtained for the distribution parameter and the sampling factor (equation [1], figure 2). There are opportunities for developing more efficient sampling plans along the following lines. The current simple random sampling plan may be changed into a two-stage random sampling plan in that the plant is sub-sampled, possibly through a random selection of stems within randomly selected plants. In this case, however, the beating tray technique is no longer satisfactory and arrangements should be made as to allow the application of destructive sampling techniques. This study, however, aimed at increasing the efficiency of the sampling plan by developing an enumerative sequential sampling plan which, in preliminary tests, appeared to be more efficient than the enumerative fixed sample size sampling plan. Nevertheless, the validity of the sampling plans and the evaluation of their efficiency should be the subject of future research work.

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