BIOLOGICAL CONTROL

Structure and Composition of the Assemblage of Parasitoids Associated to *Phyllocnistis citrella* Pupae Stainton (Lepidoptera: Gracillariidae) in Citrus Orchards in Southern Brazil

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Estrutura e Composição da Assembléia de Parasitóides de Pupas de *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) em Pomares de Citros no Sul do Brasil

RESUMO - A estrutura e a composição da assembléia de parasitóides que emergiam na fase de pupa de *Phyllocnistis citrella* Stainton, o minador-dos-citros, foram estudadas em dois pomares de citros (*Citrus deliciosa* Tenore cv. Montenegrina e *Citrus sinensis* (L.) Osbeck x *Citrus reticulata* Blanco híbrido Murcott), no Município de Montenegro (29° 68'S, 51° 46'O), RS. Em 49 ocasiões de amostragem de jul/2001 a jun/2003, todos os brotos de 24 árvores escolhidas aleatoriamente foram inspecionados. Foram registradas cinco espécies de parasitóides nativos no pomar de Murcott e seis em Montenegrina. Em Murcott, a presença de *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae), espécie exótica, foi detectada no primeiro ano de amostragem, provavelmente migrando de áreas adjacentes ao pomar onde foi liberada para o controle do minador. Em Montenegrina, sua presença foi registrada somente no segundo ano. *A. citricola* foi a espécie dominante em ambas as áreas e provocou mudanças na estrutura do complexo de parasitóides de *P. citrella* nos dois pomares.

PALAVRAS-CHAVE: Controle biológico, riqueza local, Encyrtidae, Rutaceae

ABSTRACT - The structure and composition of the assemblage of pupal parasitoids of *Phyllocnistis citrella* Stainton, the citrus leafminer, were studied in two citrus orchards (*Citrus deliciosa* Tenore cv. Montenegrina and *Citrus sinensis* (L.) Osbeck x *Citrus reticulata* Blanco hybrid Murcott), in Montenegro County (29° 68'S and 51° 46'W), southern Brazil. At fortnightly samplings, from July 2001 to June 2003, all the new shoots from 24 randomly selected trees were inspected. The species richness reached five native species in the Murcott orchard, and six in Montenegrina. In Murcott, the presence of *Ageniaspis citricola* (Hymenoptera: Encyrtidae), an exotic species, was detected in the first year of sampling, probably migrating from the nearby areas where it had been released for the miner control. In Montenegrina, its presence was only registered in the second year. *A. citricola* in both areas was dominant and changed the community structure of parasitoid complex of *P. citrella* in both orchards.

KEY WORDS: Biological control, community richness, Encyrtidae, Rutaceae

The maintenance and promotion of biological diversity should be taken as a priority for the management of sustainable agroecosystems, especially for achieving natural biological control of introduced pests. Differences in richness and diversity of natural enemies on agroecosystems subjected to different management practices are also a rising issue. It has assumed an increasing importance since the introduction of exotic biological control agents, a worldwide current practice that can significantly affect communities of indigenous natural enemies.

Diversity indices have been utilized to describe communities in natural ecosystems and compare areas, seeking information to conserve biodiversity and detect disturbances (Oliver & Beatle 1993). Lately, agroecosystems, especially perennial crops, also have been described and compared through the use of these indices to develop management techniques that improve and sustain the local diversity (Altieri 2002).

Citrus production in Rio Grande do Sul State, Brazil, is based upon small orchards, 2.0 ha to 3.0 ha on average, mainly organically grown, and under familiar management (Brasil 1999). Nevertheless, even in the organically grown orchards, the perennial characteristic of the citrus plants, together with the favorable climatic, soil and associated vegetation conditions, encourage the occurrence of several species of phytophagous insects that may become pests, decreasing yield (Koller 1994).

The citrus leafminer, *Phyllocnistis citrella* Stainton, native of Southeast Asia, and nowadays dispersed in all citrus producing regions, is one of the most important pests of this crop. It may cause direct and indirect damage to plants, reducing photosynthetic area and causing injuries that facilitate the infection by the bacteria *Xanthomonas citri* pv. *citri* (Hasse), the citrus canker causal agent (Heppner 1993, Chagas & Parra 2000).

Hymenopterous parasitoids belonging to Eulophidae, Chalcididae, Eupelmidae and Encyrtidae are the most important natural enemies recorded for *P. citrella* worldwide (Legaspi *et al*. 1999, Ateyyat 2002, Urbaneja *et al.* 2003). In Rio Grande do Sul, various Eulophidae species were registered parasitizing the citrus leafminer (Janhke *et al*. 2005, Janhke *et al.* 2006).

The encyrtid parasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) has been largely employed in programs of biological control of *P. citrella* in many countries, including Brazil (Argov & Rössler 1996, Pomerinke & Stansly 1998, Paiva *et al*. 2000). Although the introduction of this microhymenopterous may result in an increase of parasitism rate, in most cases, there is neither an evaluation of richness and community structure of native parasitoid prior to introduction, nor an assessment of the changes in these communities after the exotic agent introduction.

Hymenopterous parasitoid communities are largely employed as biological indicators in ecosystem inventories, due to their large species richness and taxon diversity that promptly react to environmental changes (Lewis & Whitfield 1999). The comparison of diversity measures in different places over time may help to elucidate the origin of the local diversity and the most suitable way to preserve it (Purvis & Hector 2000).

The present study aimed to better understand the diversity, structure and composition of *P. citrella* parasitoid community and their action upon *P. citrella* populations over time, providing information that may be used for management and control of this species.

Material and Methods

The study was carried out from July/2001 to June/2003, in Montenegro County (29° 68'S and 51° 46'W), Rio Grande do Sul State, Brazil, located in the region called "Depressão Central do Rio Grande do Sul" at an altitude of 31 m.

Samplings were carried out in two adjacent organically grown orchards, one of the *Citrus deliciosa* Tenore cv. Montenegrina and the other of the *C. sinensis* (L.) Osbeck x *C. reticulata* Blanco hybrid Murcott*,* In each orchard the sampling was done in an area of approximately 0.6 ha, containing 315 trees.

Every two weeks, 12 and 24 trees/orchard, respectively, in the first and in the second year, were randomly selected, and all new shoots of these trees were inspected seeking leaves with *P. citrella* pupae. All the leaves with pupal chamber were picked, placed in identified plastic bags and transported in icebox to the laboratory. The leaves were kept individually in sealed petri dishes, in laboratory conditions, until the emergence of parasitoids or of *P. citrella*, thus evaluating the parasitism of pupae and pre-pupae stages.

The parasitoids were identified with the aid of dichotomic keys (Penteado-Dias *et al.* 1997, Shauff *et al.* 1998) or with the assistance of Dr. Valmir A. Costa (Instituto Biológico de Campinas, São Paulo, Brazil) and MSc. Patricia A. Diez (PROIMI-Biotecnología, Argentina).

Assemblages of parasitoids that emerge in the host pupal phase were described by their species richness (S) and absolute and relative species abundance. The diversity indices of Simpson ($\lambda = \Sigma_{pi2}$), which evaluate species dominance, and of Shannon-Wiener ($H' = \Sigma_{pi} \ln_{pi}$), which estimate the species equitability, were calculated according to Moreno (2001), where p_i is the proportion of individuals in the ith species. The diversity indices of the two orchards where compared by the Bootstrap procedure that is based upon the p_j, the proportion of sample units that have species *j present*.

Alfa diversity was calculated by the rarefaction method, which calculates the expected number of species in a standard size sample, allowing the comparison of the diversity between years and the two orchards, through the formula: E (S) = χ^1- [(N –N_i)/n,] · $(N/n)^{-1}$, were: E(S) = expected number of species; N = total number of individuals in the sample; Ni = number of individuals in the ith species; n = standardized sample size. This method gives a column of abundance for a number of taxa and estimates how many taxa can be expected in a sample with a smaller number of individuals. With this method, you can compare the number of taxa in samples of different sizes (Moreno 2001).

Cumulative species abundance curve, rarefaction curve, diversity indices and comparison tests were calculated with the softwares Past 1.15 and ws2m MFC Application 2001.

Results and Discussion

The total of 498 individuals of microhymenopterous parasitoids were collected in the Montenegrina orchard and 212 in the Murcott, all belonging to Eulophidae and Encyrtidae.

In a simultaneous study carried out in the same areas, parasitism contributed 10.7% in average to the mortality of immature stages of the citrus leafminer (Jesus 2005).

Eulophidae encompass the largest number of species attacking the leafminer worldwide (Urbaneja *et al.* 1998). In Brazil, Eulophidae is represented by ectoparasitoids idiobionts that were encountered parasitizing *P. citrella* in various states, such as São Paulo (Montes *et al*. 2001, Sá 2000), Rio de Janeiro (Nascimento *et al*. 2000) and Santa Catarina (Garcia *et al*. 2001).

In the present study, the most frequently observed species belong to two subfamilies: Eulophinae and Tetrastichinae. In Eulophinae, we found *Sympiesis* sp. (Eulophini), *Elasmus phyllocnistoides* Diez, Torréns & Fidalgo and one species of *Elasmus* not identified (Elasmini), *Cirrospilus floridensis* Evans and *Cirrospilus neotropicus* (Diez & Fidalgo) both Cirrospilini. Tetrastichinae was represented by *Galeopsomyia fausta* LaSalle (Tetrastichini). The presence of *A. citricola*, an exotic parasitoid introduced in the region for the biological control of *P. citrella*, was also recorded.

The cumulative number of species stabilized along sampling, what can be observed in the curves of Fig. 1, indicating that the number of species collected reflects the actual number present. The greatest abundance obtained in Montenegrina reflects the larger vegetative growth of this cultivar, which has a denser crown when compared to the hybrid Murcott (Spósito *et al*. 1998, Rodrigues & Dornelles 1999). In fact, in the same period, a greater density of *P. citrella* was registered in the cultivar Montenegrina (Jesus 2005).

Both curves, species cumulative along the sampling curve, and species cumulative relative to the sampled individuals (Fig. 1), show that the maximum number of species present was reached at the 17th sampling occasion, and in the second year only one new species was registered in the Montenegrina area. The species richness of the citrus leafminer parasitoid community, found in citrus orchards in other Brazilian regions, varies from three to nine (Penteado-Dias *et al*. 1997, Costa *et al.* 1999, Nascimento *et al*. 2000). The number of native species varied between five and six in the areas sampled in the present study, restricted to only one family.

Sympiesis sp. was found only in Montenegrina and solely in the first sampling year (Fig. 2a). In the first year, *A. citricola* was not recorded in the cv. Montenegrina orchard, but in the Murcott orchard it was found with a relative frequency of 45% (Fig. 2). In the second year, the recording of the exotic species in the Montenegrina area corresponded to a great change in the relative frequency of the other species (Fig. 2b). Simultaneously, the increase in the number of individuals of *A. citricola* in the Murcott area occurred at the same time that a decrease in the number of some native species was observed (Fig. 2c, d).

Although the relative frequency of species changed, the absolute values of the native species did not show noticeable alterations. This could be explained by the increase in the sample size, considering that in the second year the number of sampled trees was raised.

The results suggest that after the introduction of *A. citricola* the number of individuals of the native species and the parasitoids community structure of *P. citrella* was altered. The structural change becomes clear when considering the variation in the Simpson and Shannon indices (Table 1).

The reduction in equitability in both areas from the first to the second year was indicated by the decrease on the Shannon index. Consequently, there was an increase in the dominance (lesser values of the Simpson index) caused by the increased population of *A. citricola*, which became the dominant species in both Montenegrina and Murcott orchards. The comparison of the diversity indices between areas in the same year did not reveal significant differences according to Bootstrap procedure ($P > 0.1$).

The bootstrapping method could not be used for comparison of the orchards diversity indices between years, since there was a difference in the sampling effort, which was larger in the second year, and the method assumes equal sampling conditions. Thus, the rarefaction method was used and, in the Murcott orchard, despite the difference in diversity between the years, originated by the increase in the sampling effort, there was no significant difference due to the deviations overlap (Fig. 3a).

For the Montenegrina orchard, the deviations clearly differ, indicating a lower diversity in the second year assuming a 95% confidence interval (Fig. 3b). The rarefaction

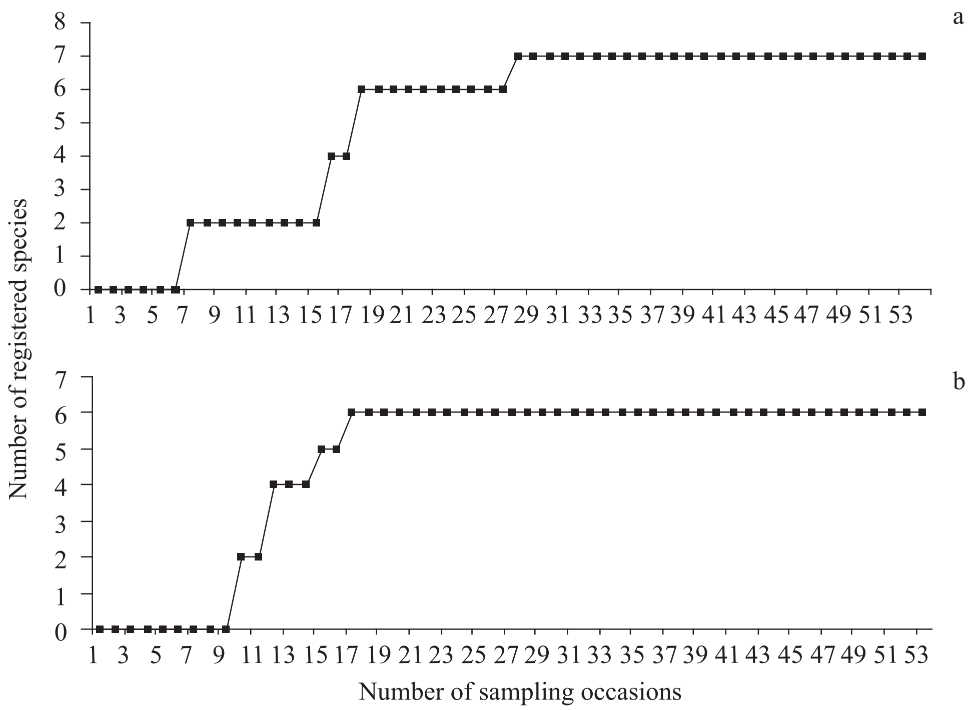

Fig. 1. Cumulative number of species of parasitoids of *P. citrella* in successive samplings of citrus orchards of Montenegrina (a) and Murcott (b) from July/2001 to June/2003, Montenegro County (29° 68'S and 51° 46'WGR), RS, Brazil.

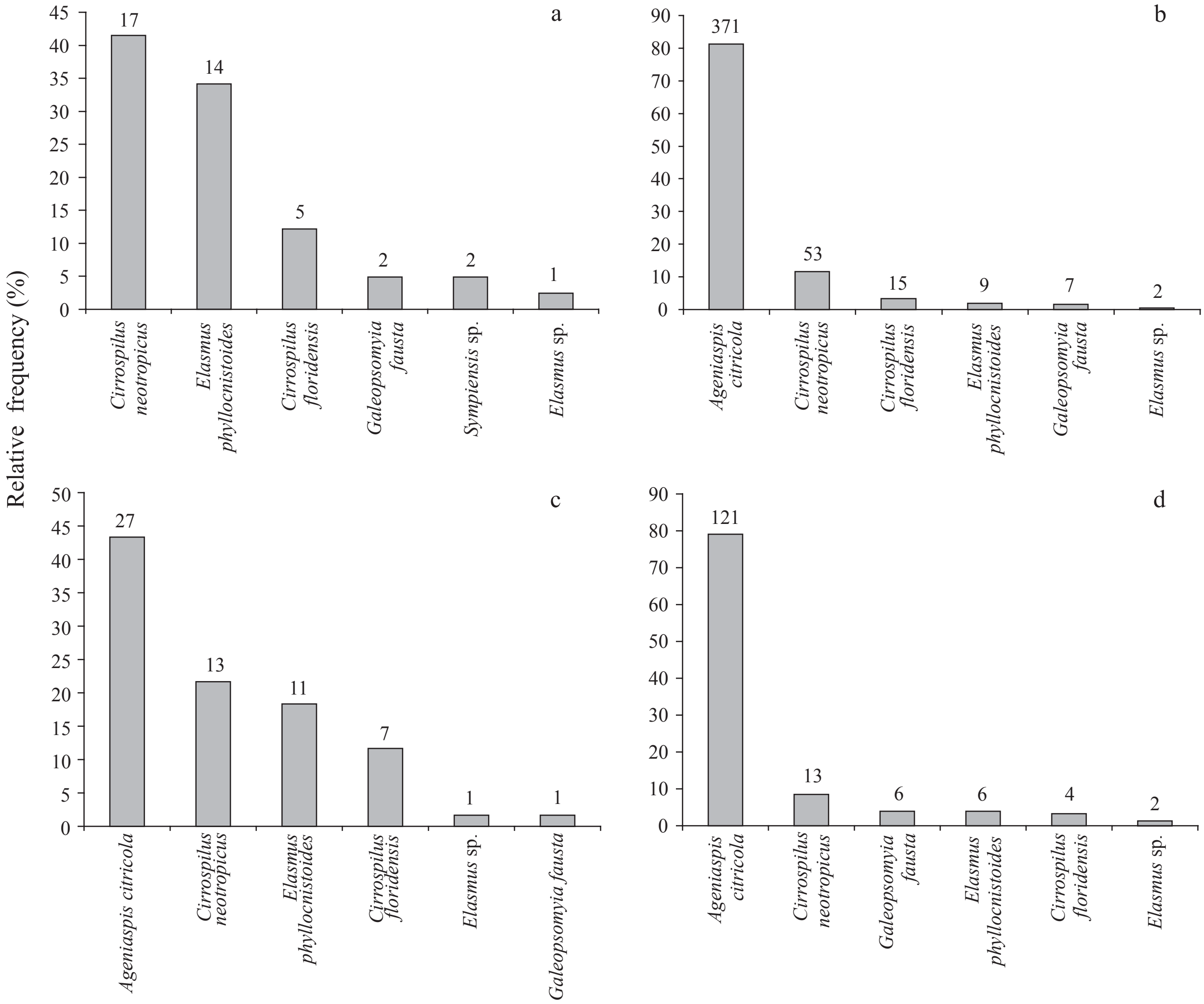

Fig. 2. Relative frequency and absolute number of individuals (in top of columns) of each parasitoid species sampled in Montenegrina in the first (July/2001 to June/2002) (a), in the second (July/2002 to June/2003) (b) and in Murcott in the first (July/2001 to June/2002) (c) and in the second year of sampling (July/2002 to June/2003) (d), Montenegro County (29° 68'S and 51° 46'WGR), RS, Brazil.

curve also supports the difference in equitability between years in Montenegrina. This difference is due to the increase of *A. citricola* population in the second sampling year.

The comparison between the orchards, regarding the entire sampling, also did not show differences (Fig. 3c), probably due to the overlapping of the deviations of the curves. These data indicate that the substrata utilized by the host did not affect the parasitoid richness, considering that most species were common to both cultivars. It seems that the presence of the exotic agent was in fact responsible for changes in the diversity, more than the availability of the substrate utilized by the host. This fact was most noticeable in the Montenegrina area, where, in the first year, the exotic species was not present. Sá *et al.* (2000) also found differences in the relative frequency of native species, as well as the absence of some less frequent species, after the introduction of *A. citricola* in citrus orchards in São Paulo State, Brazil.

The richness measures little revealed about the effect of the introduction of the exotic parasitoid in both areas, although the comparison of the beta diversity showed differences especially when dominance or equitability were considered.

Lewis & Whitfield (1999), studying the diversity of braconids in different forest management systems, found that changes in the number of common and rare species indicate environmental disturbances that alter the community structure. According to their results, these changes were more clearly perceived when patterns of relative abundance and complementary measures to diversity were considered.

In the present study, the use of different richness and diversity indices showed that the presence of the exotic parasitoid changed significantly the composition of the parasitoid complex associated with *P. citrella*, chiefly regarding the dominance structure. As indicated by

Table 1. Number of species (S), number of individuals (N) and indices of Shannon-Wiener (H') and Simpson (λ) in Montenegrina and Murcott orchards in the first year (July/2001 to June/2002), second year (July/2002 to June/2003) and in the total sampling period, Montenegro County (29° 68'S e 51° 46'WGR), RS, Brazil.

	Montenegrina			Murcott		
	1st year	2nd year	Total	1st year	2nd year	Total
S	6	6	7	6	6	6
N	41	457	498	46	140	186
H'	1.374	0.6964	0.8866	1.118	0.5757	0.7715
λ	0.6913	0.3258	0.4207	0.5992	0.2479	0.3612

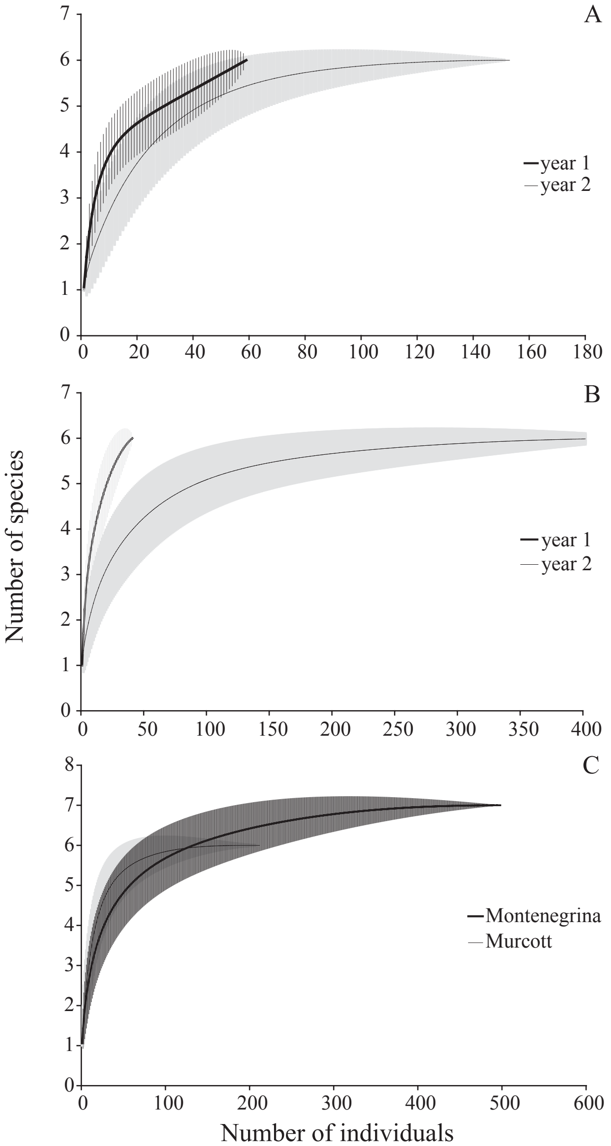

Fig. 3. Rarefaction curves and standard error to compare the species richness between the first and the second sampling year in the Murcott area (a), in the Montenegrina area (b) and the total values of richness considering both areas (c) Montenegro County (29° 68'S and 51° 46'WGR), RS, Brazil.

Drinkwater (1995), the agroecosystems are less stable than the natural ecosystems due to their smaller diversity caused by disturbances originating from inadequate management practices, by the maintenance of the system in an immature stage and by the introduction of exogenous taxa.

According to Hoy & Nguyen (1997), a monitoring program at least three years long is necessary for a clear evaluation of the changes in communities' richness and diversity as well as to estimate the actual impact of the introduction of an exotic agent. Only after that period a definitive judgment about the success or not of the introduction can be done. To the same extent, Lewis & Whitfield (1999) stated the need of using more than one sampling method to have a holistic appraisal of the parasitoid complex present in the system.

The differences detected in the orchards indicate that the presence of the exotic agent caused changes in the structure of the community of native parasitoids. Thus, the introduction of this or any other control agent should be carefully evaluated, considering that the detection of changes in diversity, relative either to the number of species or the distribution of abundance may be a signal of an environmental impoverishment (Magurran 1988).

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