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ABSTRACT — Several species of the predatory mite family Phytoseiidae are of major economic importance for biological pest control in crops, including grapevines. Plant diversification in agrosystems is reported to enhance useful biodiversity and provide ecosystem services. Thus, agroforestry, which consists in co-planting trees and crops, is assumed to be a possible way to ensure regulation of pest outbreaks by phytoseiid mites. This paper investigates the effect of trees (Pinus pinea and Sorbus domestica) within vineyards on Phytoseiidae communities. Five experimental plots were considered, two where vines were co-planted with P. pinea and S. domestica, and three monoculture plots: vines, P. pinea and S. domestica. Sampling was carried out on vines and trees in 2003, 2004, 2005, 2010 and 2012. A higher Phytoseiidae diversity was observed in agroforestry plots than in monoculture plots. Kampimodromus aberrans (replacing T. [T.] exhilaratus) seemed to colonize these plots 15 years after its plantation, especially when vines were co-planted with P. pinea. Factors affecting these faunistical changes are discussed. Effects on Phytoseiidae densities differed depending on grape cultivar-tree species associations. Factors affecting these interactions are discussed; plant diversity does not simply lead to a higher density and diversity of natural enemies.

KEYWORDS — Pinus pinea; Sorbus domestica; Vitis vinifera; Agro-Ecology; Phytoseiidae

INTRODUCTION

Relationship between biodiversity and pest control is of major interest for sustainable crop production in a framework of agroecological researches (Philpott 2013; Crowder and Jabbour 2014). In several meta-analyses (i.e. Poveda et al. 2008; Letourneau et al. 2011; Veres et al. 2013), herbivore suppression, enemy enhancement, and crop damage suppression effects were significantly associated with diversified crops. The main ecological mechanisms supporting such outcomes are (i) an effect of crop diversification on microclimate and crop physiology and then on associated fauna community (ii) the resource concentration hypothesis that states that in diversified crops, specialized herbivores have less concentrated and unlimited food supply than in monocultures and (iii) the natural enemies hypothesis that states that in diversified crops natural enemy abundance and diversity are higher because of more continuous and diversified resource availability (preys, pollen, nectar, refugia, microhabitats) compared to monocultures (i.e. Root 1973; Altieri and Whitcomb 1979; Stamps and Linit 1998; Altieri and Nicholls 2002; Tscharntke et al.
that vine agroforestry management, i.e. protection (Ratnadass et al. 2006; Liguori et al. 2001; De Villiers and Pringle 2011; Dhiel et al. 1998; Kreiter et al. 1998; Lozzia and Rigamonti 1998; Tixier et al. 2000, 2002; Zacarias and Moraes 2002; Kreiter et al. 2005; Tixier et al. 2006a). Plant diversity within plots is also assumed to affect diversity and Phytoseiidae density, some herbaceous and flowering plants being favourable to these predators (Flaherty 1969; Coli et al. 1994; Lozzia and Rigamonti 1998; Tixier et al. 1998; Kreiter et al. 2000, 2002; Nicholls et al. 2001; De Villiers and Pringle 2011; Dhiel et al. 2012; Moura et al. 2013). The hypothesis herein tested is that vine agroforestry management, i.e. combining trees and vines in a same field, could enhance biological control, by positively affecting Phytoseiidae communities in a framework of agroecological crop protection (Ratnadass et al. 2012). Effects of agroforestry on fauna biodiversity have not been fully studied yet. Impact of agroforestry in viticulture on Phytoseiidae density and diversity remains particularly poorly investigated. The studies carried out in the county Restinclières, Hérault, France constitute a major contribution with a long-term dispositive in which vine is co-planted with two trees (Pinus pinea L. or Sorbus domestica L.) (Barbar et al. 2005, 2006; Liguori et al. 2011). The present paper aims to present the most recent results obtained on Phytoseiidae communities in these plots (2012) as well as a synthesis of the previous surveys carried out for 10 years (Barbar et al. 2009; Liguori et al. 2011). The two main questions herein assessed at different time scales are: (i) Does agroforestry management affect Phytoseiidae density and diversity? (ii) Does agroforestry impact differ according to vine cultivars and co-planted tree species?

**Materials and Methods**

**Studied plots**

The plots studied are located in the Hérault, Restinclières, located at 15 kms North of Montpellier (Prades-le-Lez, Hérault, France, 43°43’2.84”N, 3°51’35.95”E). Climate is sub-humid Mediterranean and soil is predominantly clay and limestone, shallow and very stony (Dupraz 2002; Barbar et al. 2006). Plots, planted in 1997 in a fallow land dating back 35 years (Dupraz, pers. comm.), are very close to each other (maximum 100 meters distant) in an area of less than 0.2 km² within the same climatic conditions and with same uncultivated neighbouring areas predominantly composed of Pinus halepensis Miller, Quercus ilex L., Rosmarinus officinalis L., Rubus sp. and Viburnum tinus L.

Five plots corresponding to the following modalities were considered: monocultural vine, vine co-planted with *S. domestica*, vine co-planted with *P. pinea*, monoculture of *S. domestica* and monoculture of *P. pinea* (Figure 1). In each vine plot (co-planted or not), two cultivars (Syrah and Grenache) were sampled. All vine plots considered (agroforestry managed and not) were similarly managed and treated with pesticides (6-8 chemical treatments per year to control downy and powdery mildews and Scaphoideus titanus Ball). The current fungicides used were tetraconazole, benalaxyl, mancozeb, metiram, pyraclostrobine, copper and tebuconazole and the only insecticide each year used (for controlling *S. titanus*) was chlorpyrifos ethyl. The pesticides applied were chosen for their low side-effects on Phytoseiidae and no acaricide was used for 15 years (http://e-phy.agriculture.gouv.fr/). Because co-planted trees are inside the cultivated vine
FIGURE 1: Localisation of the five experimental plots (monoculture of vine, *Sorbus domestica*, *Pinus pinea* and co-planted vine with these two trees) in the county land of Restinclières (Hérault, France).
plot, they received the same amount of pesticides as vine. The monoculture tree plots were untreated.

**Samplings**

Three to ten surveys per year were performed on the following dates: 2003 (29/IV, 27/V, 24/VI, 22/VII, 12/VIII), 2004 (27/IV, 18/V, 8/VI, 12/VII, 17/VIII, 7/IX), 2005 (10/V, 12/VII, 01/IX) 2010 (5/V, 18/V, 1/VI, 28/VI, 8/VII, 17/VII, 10/VIII, 15/IX) and 2012 (2/V, 15/V, 29/V, 12/VI, 26/VI, 10/VII, 24/VII, 7/VIII, 21/VIII, 11/IX). Because of a great heat wave in 2003 the densities remained low in 2004 and 2005. Thus, we wait for five years to explore agroforestry effects and carried out new surveys in 2010 and then in 2012.

The sampling unit was a leaf for grapevine, a composed leaf for *S. domestica* and a shoot (10 to 15 cm long) for *P. pinea*. The number of vine leaves (by cultivar), *S. domestica* composed leaves and *P. pinea* shoots taken at each date and for each plot ranged between 25-100, 15-100 and 15-120, respectively depending on the year. At the beginning of the surveys (2003-2005) the numbers of leaves sampled were higher because of low densities. Then after conducting statistical analyses to determine the optimal sample size, the number of leaves sampled decrease.

Leaves and shoots were placed in separate plastic bags and then brought back to laboratory in cooler boxes. Each leaf of grape and *S. domestica* was individually observed using a binocular microscope (× 20 magnifications) and Phytoseiidae were counted and collected for mountings and identification. For statistical analyses, each leaf was considered as one replicate. Mites were extracted from *P. pinea* shoots using the “soaking-checking-washing-filtering” method (Boller 1984) considering all the shoots together and not individually because of the difficulty to observe and retrieve mites directly on pine needles. As the number of Phytoseiidae was determined for an entire sample of *P. pinea*, no statistical analysis was applied for this modality.

Only Phytoseiidae were counted and not the prey. Phytoseiidae present in French vineyards are actually generalist predators able to feed on other mites but also on insects, pollen, fungi, etc. (McMurtry and Croft 1997; Kreiter et al. 2000; Lorenzon et al. 2012). No relationship between spatial distribution of these generalist predators and Tetranychidae is usually observed (Slone and Croft 2001; Stavriniides et al. 2010). Furthermore, during all the experimental years, no damage of phytophagous mites was observed and their densities have remained very low.

**Mite identification**

The collected Phytoseiidae were mounted on slides in Hoyer’s medium and identified using a phase and interferential contrast microscope (Leica DMLB, Leica Microsystèmes SAS, Rueil-Malmaison, France) (× 400 magnifications). Females were identified using specific keys (e.g. Tixier et al. 2013) and according to the nomenclature of Chant and McMurtry (2007). The study of Tixier et al. (2006b) was used to differentiate between *Typhlodromus* (*Typhlodromus*) exhilaratus Ragusa and *Typhlodromus* (*Typhlodromus*) phialatus Athias-Henriot. Slides are deposited in the mite collection of Montpellier SupAgro (UMR CBGP) and data compiled in a database.

**Statistical analyses**

Statistical tests were performed using R Statistical Software (R Core Team 2012). To compare Phytoseiidae densities (mean number of Phytoseiidae/leaf) between the different modalities and between vine varieties, a generalized linear model based on a quasi-Poisson distribution was used (McCullagh and Nelder 1989; Ver Hoef and Boveng 2007). Then an ANOVA-test adapted to generalize linear models was performed (Hastie and Pregibon, 1992; R Core Team, 2012). Finally, the contrasts method was used through the “esticon” function (Højsgaard et al. 2012) and corrected by the Holm-Bonferroni method (Holm 1979).

**RESULTS**

**Species of Phytoseiidae**

During all surveys, three species were mainly observed: *T. (T.) exhilaratus*, *T. (T.) phialatus* and *Kampimodromus aberrans* (Oudemans). *Typhlodromus (T.) exhilaratus* globally prevailed from 2003 to 2012 in monoculture of vine, in co-planted vine with *S. domestica* and on *S. domestica* and *P. pinea* co-planted
with vine (Figure 2). In this latter modality, *K. aberrans* represented 24 % of the identified mites in 2010 and 8 % in 2012. In monoculture of *S. domestica* and *P. pinea*, *T. (T.) phialatus* globally prevailed from 2003 to 2012 (Figure 2). *Typhlodromus (T.) exhilaratus* was the second most abundant species and only one specimen of *K. aberrans* was collected in 2012.

### Densities of Phytoseiidae on grapevine co-planted or not

Phytoseiidae densities on grapevine varied between years and within a same year (Figures 3, 4). They were globally very low in 2003, 2004 and 2005 (averages of 0.12, 0.05 and 0.21 Phytoseiidae / leaf [Ph / l], respectively) and higher in 2010 and 2012 (0.54 and 0.48 Ph / l, respectively).

For all years, Phytoseiidae densities on Syrah (0.44 Ph / l) were significantly higher than those on Grenache (0.19 Ph / l) \( (P < 0.001) \) independently of the agroforestry modality tested (Figures 3, 4). This is certainly explained by different leaf characteristics and as these features can greatly affect Phytoseiidae density (Syrah with pubescent leaves / Grenache with glabrous leaves), suggesting that vine leaf characteristics, in the present case studied, more affect Phytoseiidae densities than agroforestry modality. Thus to accurately identify the effect of agroforestry management, separate analyses have been carried out for each grape vine cultivar.

**Syrah cultivar.** In 2003, densities were on the overall year lower on vine co-planted with *P. pinea* than on the two other modalities \( (P = 0.0001) \). Such difference was observed only in spring. In 2004, the opposite is observed with significant higher densities on co-planted vine with *P. pinea* \( (P = 0.0001) \), essentially early in spring. However the densities compared were very low (Figures 3a, 4). In 2005, no difference in Phytoseiidae densities between the three agroforestry modalities was observed \( (P = 0.13) \). In 2010, densities were significantly higher in monoculture vine than in the co-planted plot with *P. pinea* on 1st June \( (P = 0.049) \) and 8th July \( (P = 0.018) \) whereas throughout 2010, no significant difference was observed between the three modalities \( (P = 0.19) \). In 2012, densities in co-planted vine plots with *P. pinea* were higher than in the other two plots cumulatively throughout the year \( (P = 0.041) \), on 2nd May \( (P = 0.002) \), 15th May \( (P = 0.004) \) and 7th August \( (P = 0.045) \).

**Grenache cultivar.** In 2003, the same tendencies as in Syrah are observed, with lower densities on vine co-planted with *P. pinea* than on the two other modalities \( (P = 0.0001) \) essentially in spring. In 2004, as on Syrah cultivar, the densities were higher on vine co-planted with *P. pinea* than in the other modalities essentially early in spring and at the end of summer. However the densities compared were very low (Figures 3b, 4). In 2005, as for Syrah cultivar, no difference in Phytoseiidae densities between
Figure 3: Mean numbers of Phytoseiidae per leaf on two grapevine cultivars (a: Syrah, b: Grenache) from 2003 to 2012 in the county land of Restinclières in monoculture and co-planted vine plots. * indicates significant differences.
FIGURE 4: Mean Phytoseiidae mite densities per leaf on grapevine cultivars and on co-planted trees from 2003 to 2012 in the county land of Restinclières. MP: Monoculture P. pinea plot, PV: densities on P. pinea co-planted with vine, MV: Monoculture vine, VS: densities on Vine co-planted with S. domestica, VP: densities of Vine co-planted with P. pinea, MS: Monoculture S. domestica. SV: densities on S. domestica co-planted with vine.
the three agroforestry modalities was observed \( (P = 0.29) \). In 2010, densities were significantly higher in the monoculture vine than in agroforestry plots throughout the year \( (P < 0.001) \), on 18th May \( (P = 0.002) \), 1st June \( (P = 0.002) \) and 15th June \( (P < 0.001) \). Throughout the year and on 5th May, lower densities were observed in co-planted Grenache with \( P. pinea \) than with \( S. domestica \) \( (P < 0.001, P = 0.029) \). In 2012, no significant difference was found between the three modalities throughout the year \( (P = 0.20) \).

Only two significant differences were detected on the 21st August \( (P = 0.015) \) where densities on the monoculture vine plot was higher than in the agroforestry vine plots and on 11th September \( (P < 0.001) \) where the opposite was observed.

Densities of Phytoseiidae on \( S. domestica \) co-planted or not with vine

Phytoseiidae densities on \( S. domestica \) were low and often equal to zero during the five years (Figures 4, 5). However, these densities were higher in 2012 (mean of 0.34 Ph / l).

In 2003, no Phytoseiidae was observed on \( S. domestica \) in April, July and August. Cumulatively throughout the year, densities were higher in monoculture \( S. domestica \) plot (0.12 Ph / l) than in co-planted \( S. domestica \) (0.09 Ph / l) \( (P = 0.015) \). Cumulatively throughout the years 2004, 2005, 2010 and 2012, no significant difference was observed between the co-planted and monoculture of \( S. domestica \) \( (P = 0.25, P = 0.25, P = 0.06, P = 0.82) \) even if for some sampling dates, densities were significantly different on these two modalities.

Densities of Phytoseiidae on \( P. pinea \) co-planted or not with vine

Even if it was impossible to perform statistical analyses, Phytoseiidae densities were much higher on \( P. pinea \) than on \( S. domestica \) (annual means above 0.2 Ph / shoot) (Figure 4). Furthermore, for each year and each sample (except on 2nd and 29th May 2012) a higher number of Phytoseiidae was observed on monoculture trees than in co-planted ones (means respectively of 0.65 and 0.21 Ph / shoot) (Figures 4, 5).

**DISCUSSION**

**General considerations**

First, fauna modifications have occurred during ten years. While the dominant species is \( K. aberrans \) in Languedoc-Roussillon vineyards (Kreiter et al. 2000; Tixier et al. 2005), \( T. (T.) exhilaratus \) prevailed till 2010 when \( K. aberrans \) appeared only in the agroforestry plots. Plantation in a declaimed land (where viticulture was abandoned for decades) could have favour colonization of pioneer species such as \( T. (T.) exhilaratus \) (Tixier et al. 2005), known to be abundant in newly planted fields.

Second, Phytoseiidae densities were clearly different between the two vine cultivars considered, Syrah cv. being more favourable than Grenache cv. This is probably due to leaf characteristics; numerous deep domatia and hairy leaves as in Syrah cv. are known to favour Phytoseiidae survivorship and development whereas glabrous leaves as in Grenache cv. are known to have opposite effects (Castagnoli et al. 1997; English-Loeb et al. 2002; Roda et al. 2003; Sabbatini-Peverieri et al. 2009). Furthermore, plant leaf hairiness is known to affect pollen retention (Kreiter et al. 2002; Duso et al. 2004). As Phytoseiidae are generalist predators able to develop feeding on pollen (alternative food resource), the higher retention of pollen on the pubescent Syrah leaves than on glabrous Grenache leaves might have also affect the predator densities. Thus, effects of agroforestry should be separately considered for the two cultivars.

Finally, Phytoseiidae densities were low compared to what is usually observed on vines in the region considered (Kreiter et al. 2000; Tixier et al. 2005). This is probably due to the heat wave in summer of 2003 (very low hygrometry [less than 31%] associated with very high temperatures sometimes exceeding 40 °C) that have certainly caused high egg and immature mortalities, and then density falls in 2003 with evident consequences for subsequent years (Sabelis 1985; Liguori and Guidi 1990, 1995; Duso and Pasqualetto 1993). As such low densities could have drastically influenced effect of agroforestry in 2003, 2004 and 2005 greater credit will be given to results obtained in 2010 and 2012.
FIGURE 5: Mean numbers of Phytoseiidae per leaf on *S. domestica* and per shoot on *P. pinea* from 2003 to 2012 in the county land of Restinclières in monoculture and co-planted tree plots. * indicates significant differences on *S. domestica*.
Agroforestry management and Phytoseiidae densities on Syrah cultivar

Densities in co-planted vine with S. domestica and monocultural vine were similar while the association with P. pinea presented higher density especially in 2012. However, these differences were small and could vary between years as no difference was observed in 2010. Thus future samplings would be required to confirm a positive effect of the presence of P. pinea. This slight positive effect in 2012 could be due to higher Phytoseiidae densities observed on P. pinea than on S. domestica. Various hypotheses could be proposed to explain why these two plants differently shelter Phytoseiidae: (i) very different foliage structure: absence of domatia on S. domestica leaves could be unfavourable to Phytoseiidae development whereas the complex architecture of P. pinea shoots could constitute favourable conditions (Barbar et al. 2006); (ii) sources of food: P. pinea shelters various food such as preys (mites of the family Tenuipalpidae) and a large quantity of pollen known to be favourable to Phytoseiidae development (Engel 1990; Barbar et al. 2006) and (iii) the mycorrhization of trees: recent studies have shown positive effect of mycorrhizal fungi on epigeal fauna including mites of family Phytoseiidae (Hoffmann and Schausberger 2012) and P. pinea trees in Restinclières are mycorrhized (Dupraz 2002).

Agroforestry management and Phytoseiidae densities on Grenache cultivar

Lower Phytoseiidae densities were observed on agroforestry vine plots than on monoculture vine plots. In addition, higher densities were observed in co-planted vines with S. domestica than with P. pinea. This observation is quite contradictory with the positive effect of co-planted P. pinea previously discussed for the Syrah cultivar. Indeed, because of the very low Phytoseiidae densities observed on S. domestica, these trees could not be considered a great reservoir of Phytoseiidae. Other effects of co-planted trees than a simple "reservoir effect" could thus be hypothesized, especially hypotheses mixing interactions between trees and vine cultivars.

First, agroforestry effects could be different because Phytoseiidae species are not the same in co-planted and monoculture vine plots. Since 2010, K. aberrans was present in the co-planted vine plots (especially with P. pinea), but was absent from the monoculture vines. This effective species was absent in all plots for most of the trial period and only appeared during 2010 and 2012. The positive effect that agroforestry can have on this important predator, especially for the combination P. pinea and vine cultivar Syrah, could be due to the preference of K. aberrans for hairy leaves (Syrah cultivar) and its well-known development on pine pollen (Duso et al. 1997; Girolami et al. 2000; Kreiter et al. 2002; Kasap 2005).

Second, S. domestica and P. pinea have different canopy volumes that could differently affect microclimate in the adjacent vines (shades, hygrometry and temperature). Onzo et al. (2010) emphasized negative impact of UVB on Phytoseiidae survival (importance of shade for Phytoseiidae). As Phytoseiidae live on the underside of the leaf, at the leaf boundary layer, microclimate variations could affect their development, as demonstrated by Walter (1996) and Stavrinides et al. (2010). It can be assumed that such effects could be different at vine leaf level depending on the cultivar considered and its hairiness.

Thirdly, P. pinea produced favourable pollen easily transported by wind (wind-pollination). Sorbus domestica is a hermaphroditic plant with insect-pollination, which furthermore produces less pollen (Oddou-Muratorio et al. 2006). It can thus be assumed that pollen of P. pinea will be more easily dumped on grape leaves than those of S. domestica. Furthermore, as discussed above, retention could be more efficient on hairy vine leaves than on glabrous ones.

Fourthly, co-planted trees could compete with vine stocks for nutrients and water. Depending on the vine cultivar but also on the co-planted trees, competition amplitude could be different. Phytoseiidae development is negatively affected by less turgescent plants (due to water stress) (Malison 1994). Stavrinides et al. (2010) also showed that water stress in vine plots is associated with leaf temperature increase resulting in a decrease of Phytoseiidae density. As vine cultivars show different re-
sponses to water stress (isotropy and anisotropy) (Pou et al. 2012), as the nature water stress is certainly different according to the co-planted tree species (and root systems), it could be assumed that these interactions will affect plant turgor and Phytoseiidae mite development. However, further integrated studies including plant physiology are clearly required to test this latter hypothesis.

Finally, mycorrhization effects could be hypothesized as previously mentioned. Mycorrhization of roots can have a positive impact on the Phytoseiidae species Phytoseiulus persimilis Athias-Henriot and negative impacts on the pest species Tetranychus urticae Koch (Hoffmann et al., 2009; Nishida et al., 2010; Hoffmann et al., 2011a, b, c; Hoffmann and Schausberger 2012).

Agroforestry management and Phytoseiidae diversity

Kampimodromus aberrans and T. (T.) exhilaratus prevailed in vine crops but were not present in monocultures of trees. These two species are currently observed in agrosystems especially in vineyards in South of Europe (Tixier et al. 2013). These two species seem thus to be adapted to agronomic practices and associated perturbations (pesticide applications, food resources, etc.) whereas the main species found on monoculture tree plots and the surrounding environment (T. [T.] phialatus) would not. Barbar et al. (2008) showed that populations of T. (T.) exhilaratus present in vineyards of Restinclières are less sensitive to chlorpyrifos-ethyl (insecticide used to control S. titanus in the studied plot) than T. (T.) phialatus found in surrounding uncultivated areas. Typhlodromus (T.) exhilaratus present in treated Italian vineyards developed resistance to fungicides and insecticides (Liguori, 1988), whereas abundant populations of T. (T.) phialatus were found in Iberian vineyards with minor or no pesticide applications (Garcia-Mari et al. 1987, Pereira et al. 2003).

The present study showed that even if other predators are present in high numbers in neighboring vegetation, they do not succeed in colonizing cultivated vine plots and co-trees immediately. Thus agroforestry can help to improve the increase of beneficial predators inside vineyard plots.

CONCLUSION

This paper provides a synthesis of the first studies assessing large-time scale impact of agroforestry on Phytoseiidae communities in vineyards. Fifteen years after establishing of agroforestry plots and ten years after intensive surveys, impact of this association seems complex and varies according to tree species and grapevine cultivars. Though co-planted trees seem to act as direct reservoirs for Phytoseiidae generalist predators, other influences were herein hypothesized and further experiments are required to test them (effect of shadow, effects of competition between vine stocks for nutrients and water on mite communities, pollen reservoir and dispersal from the trees ...). Furthermore, surveys of the Phytoseiidae community should be continued to determine if K. aberrans will successfully colonize vineyards. As well, it might also be necessary to study the effects of agronomic practices on the habitat, food source, temperature, etc., important for Phytoseiidae survivorship.

Finally, considering the widely accepted concept of the effect of diversification of agricultural systems on natural enemy diversity and densities (natural enemy hypothesis), the present study did not demonstrate an evident effect on Phytoseiidae diversification (only few species were able to develop in cultivated plots with or without co-planted trees) nor on the Phytoseiidae densities (even if tendencies are encouraging). This study also showed that natural colonization of Phytoseiidae predators from neighbouring vegetation was not so effective, though in the South of France it was (Tixier et al. 2006a). In this specific case, agroforestry seems to be the solution for the increase of Phytoseiidae predators and biological control.

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