Host-specificity and functional diversity among arbuscular mycorrhizal fungi

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi are mutualistic symbiotic associations between 150 species of fungi and the roots of approximately 300 000 species of plants. As a result of this low fungus:host species ratio, it has been assumed that the fungi are not host-specific and that there is a high functional redundancy among fungal species. In this study, fungi and plants were isolated from an old field meadow and used in a series of experiments to test for host-specificity and functional redundancy. AM fungi were not host specific - 96% of combinations yielded successful colonization. However, there was strong evidence for functional variability among fungal species. Differential responses in P uptake, protection against non-mycorrhizal fungal infection, and productivity was detected for almost every plant-fungus combination tested. The nature of the responses was highly fungus- and plant-species specific. These results suggest that changes in the species composition of AM fungal communities can strongly alter plant community structure.

Introduction

Few studies have explored the role of soil organisms, particularly mycorrhizas, in regulating the diversity of plant species or guilds. This is despite the fact that most significant ecosystem functions (ie. primary productivity, nutrient cycling, trophic patterns) are based on feedback mechanisms between above and belowground organisms, and that mycorrhizal fungi are known to act as critical mediators in many of these processes (Brundrett 1991; Francis and Read 1994).

Many plant and fungal ecologists have assumed that AM fungi are functionally redundant (Allen et al. 1995), at least with regard to plant fitness and community structure, and have ignored the possible role of AM fungal diversity in regulating floral diversity and productivity. Despite the proliferation of research on AM fungi, most of it has focused on only a handfull of plant and fungal taxa (Klironomos and Kendrick 1993) and very few researchers have examined feedbacks between the diversity of plants and AM fungi. Two studies have examined the influence of plants on AM populations and both found that different plant species stimulated different levels of AM propagation (Johnson et al. 1992; Sanders and Fitter 1992), while a unique microcosm study (Grime et al. 1987) found that plant diversity increased in response to a single AM species inoculation. A more recent study (van der Heijden et al. 1998a) showed that plant species differed in their dependancy on AM fungi and that specific fungal species had significantly different effects on plant growth. Together, these studies suggest that different AM fungi play different roles within ecosystems.

In this study we tested the hypothesis that AM fungi within a single habitat are not host-specific in their ability to successfully colonize plant roots, but that AM fungal diversity is

positively correlated with functional diversity of the mycorrhizae and that there may be little functional redundancy.

Methods

The study site was the Long-Term Mycorrhiza Research Site (LTMRS) established in 1996 at the University of Guelph (43°32'30"N, 80°13'00"W). The site was originally farmed but has not been disturbed since at least 1967. It is now considered an old-field meadow, dominated by perennial grasses, asters, and goldenrods. Seeds from the following plants were collected from the site: *Agrostis gigantea* Roth., *Bromus inermis* Leysser., *Achillea millefollium* L., *Aster novae-angliae* L., *Chrysanthemum leucanthemum* L, *Daucus carota* L., *Fragaria virginiana* Duchesne, *Plantago lanceolata* L., *Rudbeckia hirta* L. and *Solidago canadensis* L. The following AM fungi were isolated from the site using trap cultures (Brundrett et al. 1994) and maintained in pot culture on *Allium porrum*: *Acaulospora denticulata* Sieverding & Toro, *Acaulospora spinosa* Walker & Trappe, *Entrophospora colombiana* Spain & Schenck, *Gigaspora gigantea* (Nicolson & Gerdemann) Gerdemann & Trappe, *Gigaspora margarita* Becker & Hall, *Glomus etunicatum* Becker & Gerdemann, *Glomus intraradices* Schenck & Smith, *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe, *Scutellospora calospora* (Nicolson & Gerdemann) Walker & Sanders, and *Scutellospora pellucida* (Nicolson & Schenck) Walker & Sanders.

Experiment 1

The ten plant species were crossed with the ten fungal species for a total of 100 combinations which were replicated 5 times. Each pot received 15 g of leek-root inoculum and three, 1 week old seedlings. They were cultured in a completely randomized design for 4 months in the greenhouse and received a weekly fertilization of half-strength Hoagland's solution. Roots were harvested and stained using Chlorazol Black E (Brundrett et al. 1984). Compatibility was deemed positive if arbuscules or vesicles were found in the roots in at least one of the five replicates.

Experiment 2

Five plants (*B. inermis*, *A. millefollium*, *A. novea-angliae*, *P. lanceolata* and *R. hirta*) and fungi (*A. denticulata*, *G. etunicatum*, *G. intraradices*, *G. mosseae* and *S. calospora*) were crossed and set up as in the previous experiment with the addition of a non-mycorrhizal control for each plant species. After three months, two leaves were collected from each plant and analyzed for %P (Olsen and Sommers 1982). A root subsample was assessed for % colonization by arbuscules, vesicles and hyphae (McGonigle et al. 1990). Five grams of substrate was collected and extraradical hyphae were assessed (Miller et al. 1995). The remainder of the plant was dried at 60^{o}C for 48hrs to determine dry weight.

Experiment 3

This was set up exactly as the previous experiment except that all the pots were further inoculated with 1 x 10^6 conidia of *Fusarium solani* (Mart.) Sacc. isolated from the LTMRS. This fungus was found to be a weak parasite/saprobe when associated with the plants in this study. After 3 months, roots were harvested, stained with Chlorazol Black E and % infection by non-AM fungi was determined.

In experiments 2 and 3, differences between mycorrhizal treatments and non-mycorrhizal

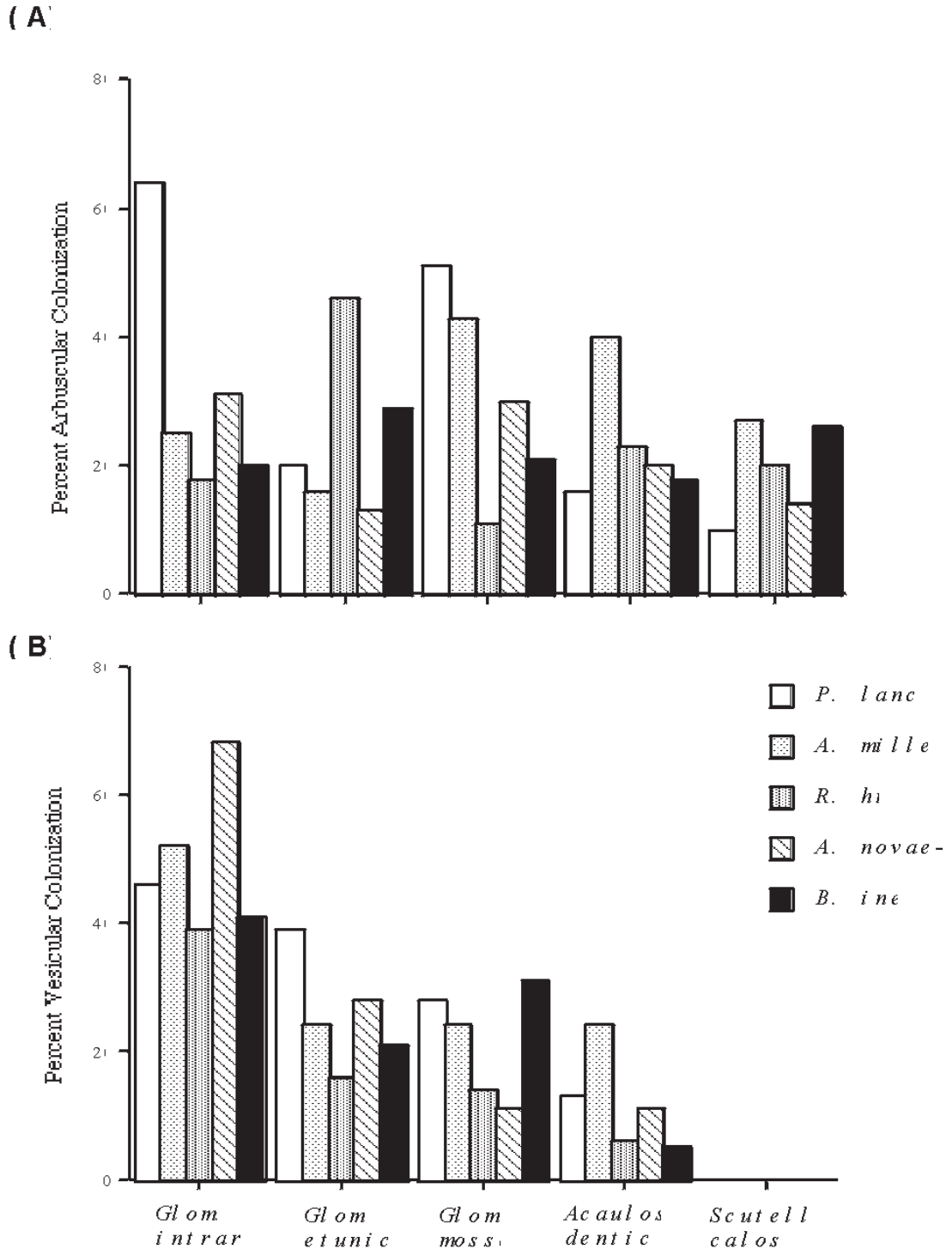
(A)
Percent Arbuscular Colonization
8
6
4
2
0
(B)
Percent Vesicular Colonization
8
6
4
2
0
P. l anc
A. mi l l e
R. h
A. novae-
B. i ne
Gl om i nt rar
Gl om et unic
Gl om moss
Ac aul os dent i c
Scut el l cal os

Figure 1.

controls were analyzed with a student t-test. Differences among plant or fungal treatments were analyzed with ANOVA (Zar 1984).

Results

Experiment 1

Ninety-six of the 100 crosses resulted in successful mycorrhizal development. The following crosses were not successful: *A. gigantea* x *A. spinosa*, *A. millefollium* x *E. colombiana*, *A. novae-angliae* x *S. pellucida* and *S. canadensis* x *S. pellucida*.

Experiment 2 and 3

Percent arbuscular colonisation (%AC) ranged from 10% to more than 60% in the 25 plant/fungal crosses tested (Fig 1a). There was no apparent plant ($p=0.43$) or fungal-species pattern ($p=0.29$). The level of arbuscular development depended on plant/fungal combination. Vesicular colonization was more consistent among fungal species ($p=0.0001$; Fig 1b). Plants colonized by *G. intraradices* produced the most vesicles (>40%), whereas those colonized by *S. calospora* did not produce any. The other fungi produced intermediate levels of vesicles. Non-mycorrhizal plants did not have either structures. Intraradical hyphal colonization differed (20%-80%), with the *Glomus* species containing the higher levels than the *Acaulospora* and *Scutellospora* species ($p=0.003$; Fig 2a). The inverse was true for the extraradical hyphal lengths ($p=0.019$; Fig 2b). Colonization increased %foliar P in most plants, but only when colonized by *Acaulospora* ($p=0.0001$) and *Scutellospora* ($p=0.002$) (Fig 3a). The 3 *Glomus* species were the only ones that significantly reduced infection by *Fusarium solani* and other non-mycorrhizal fungi ($p=0.042$; Fig 3b). Furthermore, neither of the above parameters were correlated with change in plant biomass.

Discussion

The results suggest that AM fungi are not host-specific. Only a few combinations (4%) did not match, but since the fungi tested did not represent the whole AM fungal community, there is still room for a higher degree of host-specificity. Nonetheless, this is the first report which test this hypothesis empirically using plants and fungi isolated from a single habitat compared to previous studies which tested a few cultured fungi on a variety of plant hosts (Smith and Read 1997). Despite the lack of host-specificity, these results illustrate that AM fungi differ in their intra- and extra-radical morphologies as well as their effects on plant nutrition, growth and succeptibility to infection by other soil-borne fungi. From the present results AM fungi can be separated into two functional groups; those that enhance P-uptake and those that protect against soil-borne parasites and pathogens. Even though they are not restricted to particular plant species or genera, they are very specific in the ecophysiological functions they provide and cannot be described as functinally redundant. Suprisingly, the fungi that are better able to increase P-uptake developped more extensive extra-radical hyphal lengths and were found in genera other than *Glomus*. However, this genus was more effective at preventing *Fusarium* and other non-mycorrhizal fungi from infecting host roots. Interestingly, other studies that successfully found AM fungi to protect against plant pathogens also used *Glomus* species (Perrin 1990). This study only reports on two functions. It is highly likely that other specialities exist, such as water uptake, cold tolerance, grazing resistance, among others. Furthermore, there does not seem to be any

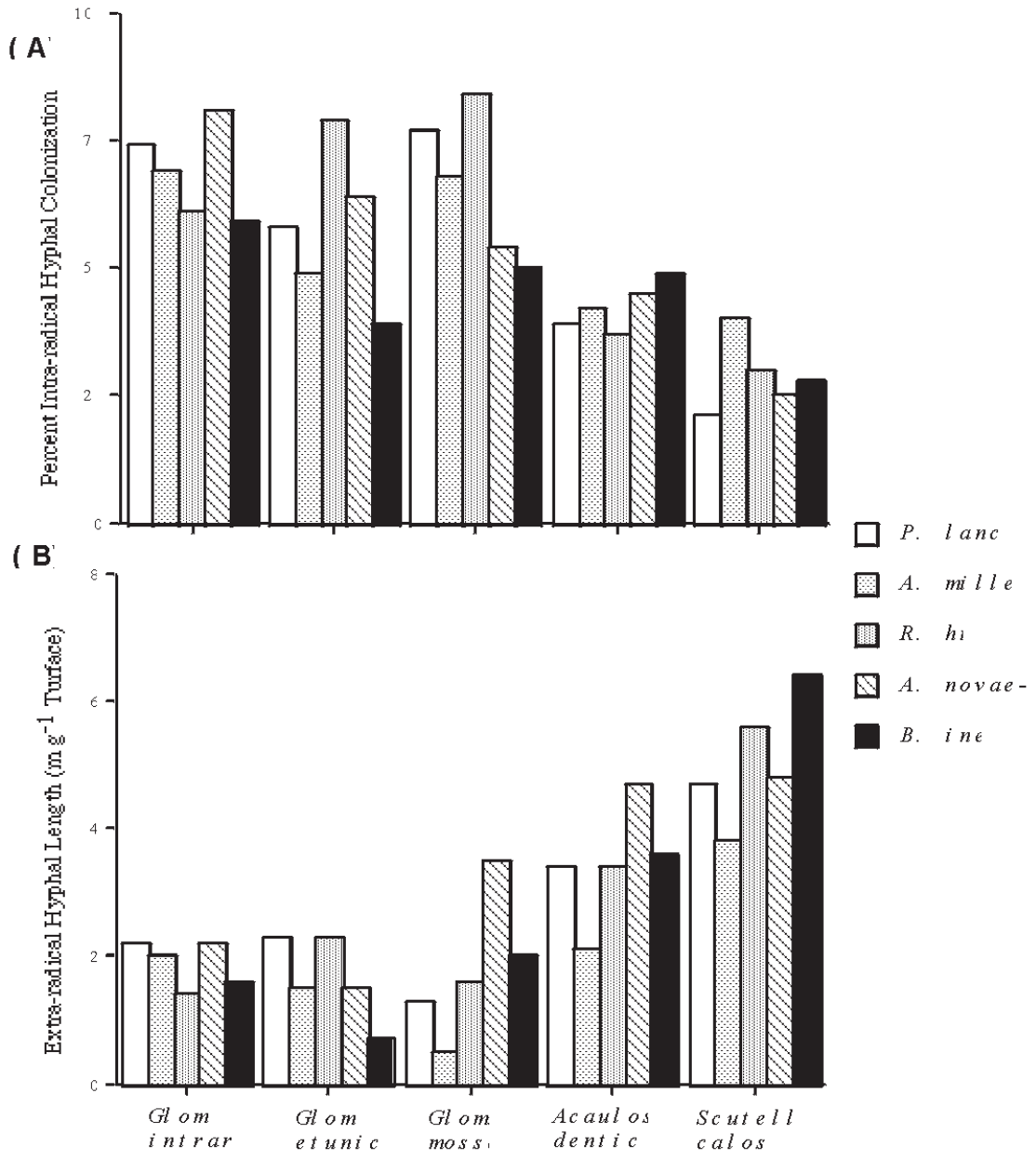

Figure 2.

relationship between percent fungal colonization, foliar P concentrations and plant growth. Although *A. denticulata* results in high foliar P concentrations, the growth response of the plants is generally smaller than with *G. mosseae*, which produces lower P concentrations. Thus, in order to better understand plant growth responses to AM fungal inoculation we must consider a variety of functions associated with the fungi, not just P-uptake.

The results also imply that through their differential effects on plants, AM fungi have the potential to determine plant community structure. Grime et al. (1987) showed that the presence of AM fungi can strongly influence plant community structure. Plants differ in

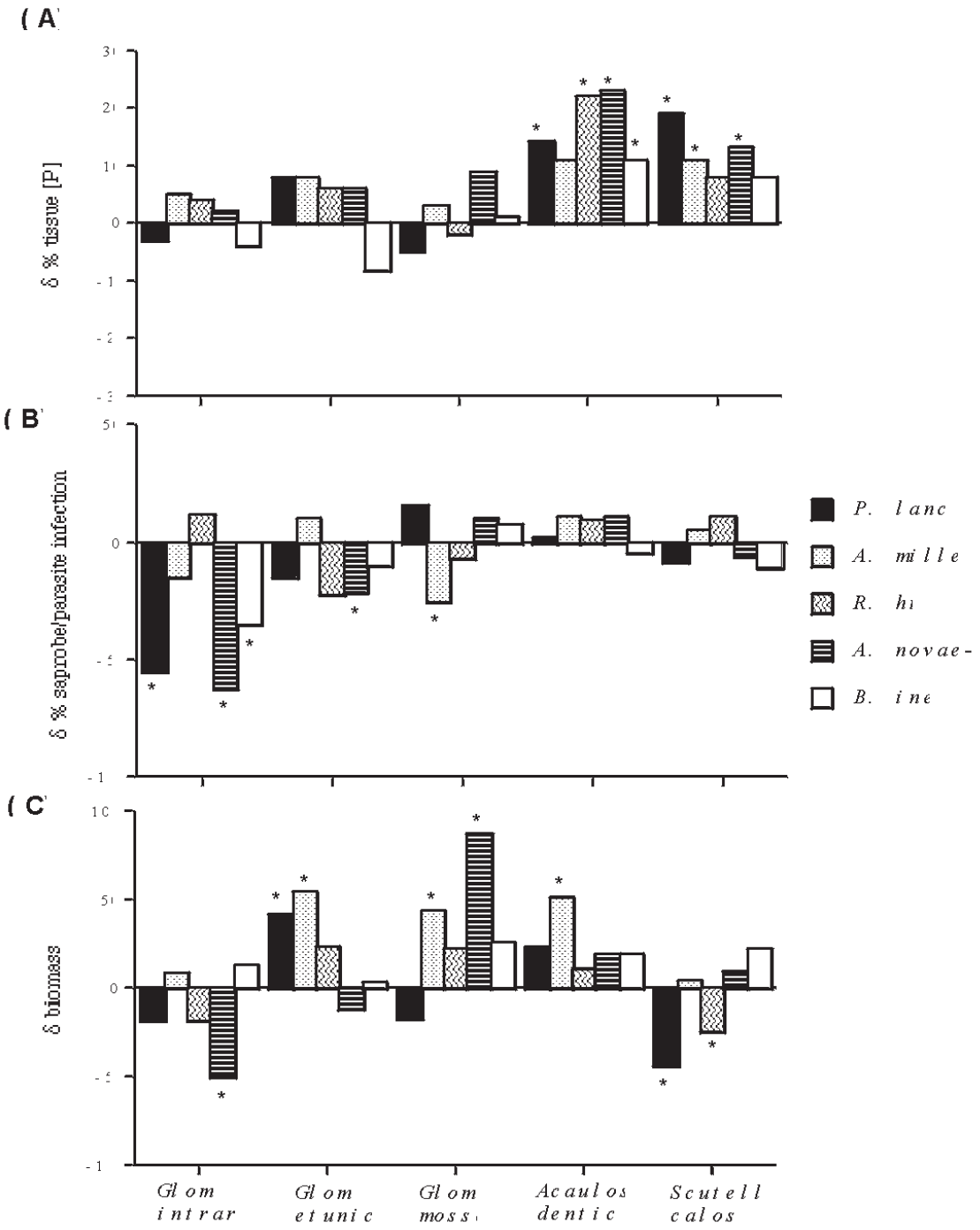

Figure 3.

their mycorrhizal-dependency and in the absence of the fungi, some cannot compete for soil resources and do not survive. A more recent study further showed that mycorrhizal dependency can vary greatly because of differential growth responses to specific AM fungi (van der Heijden et al. 1998a). In the present study, plants either increased, decreased or showed no change in biomass when colonized by different AM fungi, supporting the

previous findings and further illustrating that the end product of the symbiosis can be anywhere along the mutualism-parasitism continuum (Johnson et al. 1997) partly depending on the organisms in question. In another experiment (van der Heijden et al. 1998b), fungal diversity was manipulated and significant increases in nutrient capture, plant diversity and productivity were found in response to increasing AM fungal species richness. These results further demonstrate that AM fungi are not functionally redundant and emphasize the need to consider AM fungal diversity in the management or restoration of plant communities.

Acknowledgements

I would like to thank Margot Ursic, Peter Moutoglis and Vanessa Turner for technical assistance. This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

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