Commentary

An increasingly scented world

We know that, at least in the short term, a rise in temperature exponentially increases the emission rates of most biogenic volatile organic compounds (BVOCs). It does so by enhancing the enzymatic activities of synthesis, by raising the BVOC vapour pressure, and by decreasing the resistance of the diffusion pathway (Tingey *et al.*, 1991). BVOC emissions are thus expected to increase strongly with globally rising temperatures (IPCC, 2007). By applying the most frequently used algorithms of emission response to temperature (Guenther *et al.*, 1995), it is easy to estimate that climate warming over the past 30 yr (IPCC, 2007) could have already increased BVOC global emissions by 10%. A further 2–3°C rise in the mean global temperature, which is predicted to occur early this century (IPCC, 2007), could increase BVOC global emissions by an additional 30–45%. Furthermore, global warming in boreal and temperate environments not only means warmer average and warmer winter temperatures, but also implies an extended plant activity season (Peñuelas & Filella, 2001), further increasing the total annual emissions. The biological and environmental effects of such increases in emissions can be substantial (Peñuelas & Llusià, 2003).

However, many investigations are conducted in the laboratory, and we still do not know very much about the emissions of BVOCs in the field in response to warming. We know even less about medium- to long-term responses of BVOC emissions to warming, and about some regions of our planet, such as the Arctic, that are likely to experience the most pronounced effects of climatic warming. Tiiva *et al.* have started to fill this gap in our knowledge in this issue of *New Phytologist* (pp. 853–863). They have measured the emissions of isoprene, the most emitted reactive BVOC from vegetation, at a subarctic heath that they have experimentally subjected to a 3–4°C air temperature enhancement for 8 yr. Tiiva *et al.* have measured increases in emissions ranging between 56 and 83% depending on the year. Their results confirm the percentage increases in emissions expected using the standard algorithms of Guenther *et al.* (1995).

'Increasing production and emission of BVOCs may be largely beneficial for plants, which are likely to gain increased protection in the face of abiotic stressors …'

Increased BVOC emissions in a changing world

Warming does not only have direct effects on BVOC emissions. It has numerous indirect effects. One of these is linked to the consequent changes in land cover. In environments that have winters with freezing temperatures, an increase in minimum winter temperature of 5°C is expected to increase the number of species able to grow there by 7–20% (Niinemets & Peñuelas, 2008). In fact, as a consequence of the warming of the last few decades, migrations of the tree-line northward and upslope and increasing abundance of deciduous woody shrubs in Arctic vegetation communities have already occurred (e.g. Tape *et al.*, 2006). These vegetation changes lead to increasing amounts of leaf litter on the ground (Cornelissen *et al.*, 2007), and this, in turn, brings extra nutrients to the soil (Rinnan *et al.*, 2008). In their study, Tiiva *et al.* also tested the effects of an addition of mountain birch (*Betula pubescens* ssp. *czerepanovii*) litter during the 7–8 yr of their experiment, thus simulating the warming-induced expansion of deciduous shrub species and migration of the tree-line, and therefore an increased availability of nutrients. They did not find any significant effect on BVOC emissions. The absence of response to the litter addition does not fit the hypothesis of increased emissions under increasing litter-fall which was based on observations of increased isoprene emission under nutrient fertilization (e.g. Harley *et al.*, 1994), and on the expected increases in carbon fixation and in the activity of the enzymes responsible (Litvak *et al.*, 1996). It might be that the expected positive response is still limited by temperature in this Arctic ecosystem, and that positive effects may thus only be found in more meridional latitudes which are not energy limited. However, there have been few studies in warmer regions, and only variable and complex species-specific and compound-specific responses have been reported in meridional latitudes (Blanch *et al.*, 2007).

The shifts in species dominance and the changes in land use and cover that are currently occurring, and that are projected for the next few decades, can also affect BVOC emissions dramatically because these emissions are species-specific, and many of the plant species migrating to northern latitudes and higher altitudes are strong emitters of BVOCs such as isoprene and monoterpenes. For instance, most broad-leaved species of *Populus* or *Quercus* and, essentially, all conifers are important emitters of volatile isoprenoids (Niinemets & Peñuelas, 2008 and references therein).

Increases in emissions in response to changes in land cover are expected to occur not only in Arctic regions, but in many regions around the globe. For example, in some tropical areas the rainforest has been replaced by plantations; for instance, palm plantations in Malaysia and rubber tree plantations in southern China (Wang *et al.*, 2007). Not only do these

Global environmental changes:
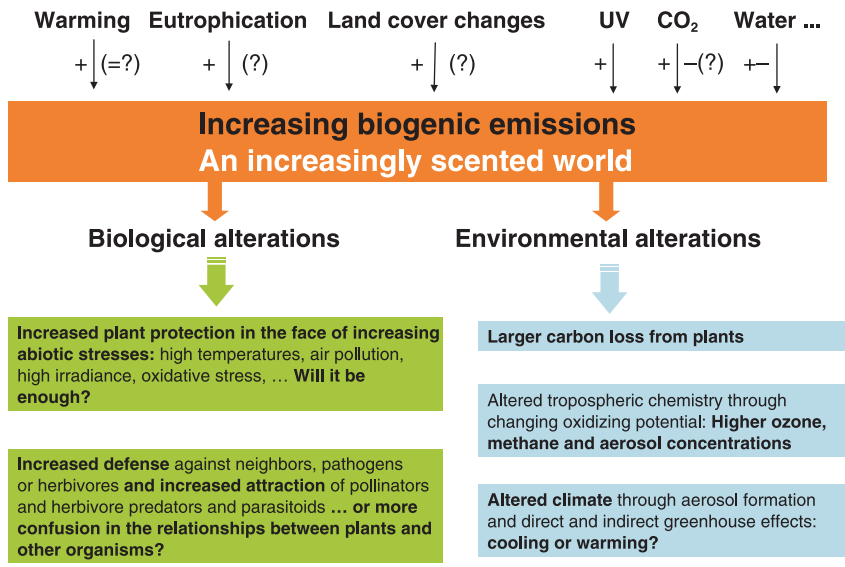
Fig. 1 Scheme of the possible effects of global environmental changes on plant volatile organic compound emission rates and the still unknown biological and environmental consequences.

plantations emit up to 10 times more isoprenoids than natural forest, but some of their emitted compounds can respond more strongly to warming (S. Owen *et al.*, unpublished; Wang *et al.*, 2007). Other land cover changes might also greatly increase BVOC emissions, for example abandonment of agricultural land in temperate regions, and subsequent afforestation with evergreens such as *Eucalyptus*, *Quercus* or *Pinus*, which are strong emitters of BVOCs throughout the year.

Warming, eutrophication and land cover changes are not the only global environmental changes that can potentially increase BVOC emissions (Fig. 1). Enhanced UV-B radiation may substantially increase emissions, as reported for the Arctic in another recent study by Tiiva *et al.* (2007). The rising atmospheric CO_2 concentrations are likely to increase the productivity and standing biomass of plants, at least in the short term, and hence also facilitate further production and emission of BVOCs. However, the number of studies on CO_2–BVOC interactions is still limited, and their results are sometimes contradictory. It is not clear whether or not elevated CO_2 *per se* increases the release of BVOCs (Peñuelas & Llusià, 2003). In fact, recent work indicates that increasing CO_2 concentration may uncouple isoprene emission from photosynthesis (the carbon source for BVOCs), and inhibit isoprene emission at the leaf level (Possell *et al.*, 2005). Changes in water availability also affect BVOC emissions. The decrease of isoprenoid emission in response to severe droughts, possibly through effects on protein levels or substrate supply (Fortunati *et al.*, 2008), might largely offset the predicted impact of rising temperatures on the emission of isoprenoids in arid and semiarid terrestrial ecosystems suffering more frequent severe droughts.

Thus, there is still a lack of precise and complete data addressing the question of what the combined effects of these components of global change, and many others that have not

been considered here, such as changing irradiance, stillness and air pollution, will be on BVOC emissions. For example, we still wonder whether or not BVOC emissions will acclimate to long-term warming. Moreover, the complex interaction of each one of these global change drivers with other biotic and abiotic factors introduces a great deal of variability into the responses to global environmental changes. However, our current knowledge seems to indicate that the most likely overall response will be an increase in BVOC emissions.

At this point, the reader may ask the question: so what? Why do we bother about these changes in the amounts of emitted BVOCs? We bother because the effects, both biological and environmental, can be far-reaching and substantial.

Biological and environmental alterations

Increasing production and emission of BVOCs may be largely beneficial for plants, which are likely to gain increased protection in the face of abiotic stressors such as the high temperatures themselves, air pollution, high irradiance, oxidative stress, or mechanical wounding. Will the degree of protection increase in proportion to the stress? This is a challenging issue to address.

The increased emissions will also affect plant communication and relationships with other organisms. Plants with increased emissions may have enhanced deterrence against pathogens or herbivores, enhanced allelopathic effects against neighbors, enhanced attraction of both pollinators and herbivore predators and parasitoids, or enhanced antimicrobial defense. If plants become more scented, we will experience a dramatically changed world in which olfactory cues are much more important as ecological and evolutionary factors. But will all these expected enhancements in defenses actually occur, or will the organisms

receiving the enhanced BVOC messages from plants be puzzled by the altered emissions? Whatever the direction of the responses, the consequences for the structure and functioning of life on our planet may be very significant.

The changes resulting from increases in BVOC emissions will not only be biological. Increased emissions will also affect the atmosphere biogeochemically and biophysically. The loss of carbon as isoprene to the atmosphere in the study by Tiiva *et al.* was in general less than 0.1% of the net ecosystem carbon assimilation at the heathland study site. However, the exponential response of BVOC emissions to temperature translates into a 3- to 6-fold increase for a 10°C rise in temperature (Q_{10} value), whereas the Q_{10} of typical biochemical reactions such as photosynthesis is only 2–3 (Niinemets, 2004). Therefore, during the periods of the highest isoprene emissions in Tiiva *et al.*'s study, the carbon loss reached 1% of the mean net ecosystem carbon assimilation rate. This amount of carbon loss is at the same level as previously measured, but BVOC fluxes can account for 5–10% or even more of total net carbon exchange, especially under stressed conditions (Peñuelas & Llusià, 2003). Therefore, these BVOC emissions may represent a significant plant carbon loss on an ecosystem basis and on a global basis. The global average BVOC emission for vegetated surfaces is 0.7 g C m^{-2} yr^{-1} but could exceed 100 g m^{-2} yr^{-1} in some tropical locations (Guenther, 2002). BVOC emissions may become an even more significant component in local and regional carbon budgets as they increase in response to global changes.

BVOCs influence the oxidizing potential of the troposphere by affecting the concentration of the main atmospheric oxidant, the hydroxyl radical. Thus, increased BVOC emissions will affect crucial features of atmospheric chemistry such as ozone dynamics, aerosol formation, carbon monoxide production and methane oxidation (Peñuelas & Llusià, 2003, and references therein) The effects will thus be multiple; for example, currently, there is only limited formation of spring or winter smog in northern and high-altitude habitats, but conditions might become conducive for smog production if warming continues.

Furthermore, these increases in the emissions of BVOCs might make a major contribution (via positive or negative feedback) to the complex processes associated with global warming itself. Until recently it was thought that the short lifetime of BVOCs would preclude them from having any significant direct influence on climate. However, there is emerging evidence that this influence might be significant at different spatial scales, from local to regional and global, through aerosol formation and direct and indirect greenhouse effects. BVOCs generate large quantities of organic aerosols (Claeys *et al.*, 2004) that could affect climate significantly by forming cloud condensation nuclei. As a result, there should be a net cooling of the Earth's surface during the day because of radiation interception. Apart from the direct local BVOC greenhouse effect, which has detectable effects only when

canopy-scale BVOC emissions are high, an additional global indirect greenhouse effect must also be considered because BVOCs increase ozone production and the atmospheric lifetime of methane, and hence enhance the greenhouse effect of both these gases. Whether the increased BVOC emissions will cool or warm the environment will depend on the relative weights of the negative (increased albedo) and positive (increased greenhouse action) feedbacks (Peñuelas & Llusià, 2003).

The study of Tiiva *et al.* thus shows increased BVOC emissions with warming in field conditions and, moreover, it reminds us of the many unanswered questions regarding the relationship between global change and BVOCs. We still do not know how much BVOC emissions will increase in response to the different global change drivers, but we know that we must focus not only on the substantial effects of climate warming on BVOC emissions, but also on the effects of the other global change drivers, especially increasing changes in land cover. The expected increases in BVOC emissions are likely to have far-reaching biological and environmental effects, warranting interactive interdisciplinary research by biologists, physicists and chemists at foliar, ecosystem, regional and global scales.

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# Getting sick may help plants overcome abiotic stress

In their environment, plants establish relationships with many microorganisms such as fungi, bacteria and viruses which can be either pathogens or symbionts. Common wisdom states that mutualistic/symbiotic associations are beneficial to the plant but pathogen associations deleterious. However, recent papers in *New Phytologist* by Xu *et al.* (this issue; pp. 911–921) and Baltruschat *et al.* (2008; issue 180 (2); pp. 501–510) underline the point that this view is perhaps too simplistic. In the case of pathogenesis, one possibility for the plant to prevent or minimize microbe infection is to generate an oxidative burst, the purpose of which is to kill bacteria and plant cells surrounding the infection site. However, recent data indicate that reactive oxygen and nitrogen species (ROS and RNS, respectively) are produced by both partners in many symbiotic and pathogenic systems (Delledonne *et al.*, 2001; Tanaka *et al.*, 2006; Baptista *et al.*, 2007; Jones *et al.*, 2007; Molina & Kahmann, 2007; Shetty *et al.*, 2007). Therefore, in a pathogenic or symbiotic association, both the plant and the microbe must be able to deal with a complex mixture of ROS coming from both sides. ROS are not necessarily harmful for the partners and, depending on the model considered, they can also help to signal and limit/control the interaction. For example, the development of a mutualistic association between *Epichloë festucae*, a fungal endophyte, and the grass *Lolium perenne* requires the production of superoxide or hydrogen peroxide by a fungal NADPH oxidase, whilst inactivation of this gene changes the interaction from mutualistic to antagonistic (Tanaka *et al.*, 2006). In any case, both partners (the plant and the microbe) have developed an impressive array of nonenzymatic and enzymatic antioxidant systems, whose function is to maintain adequate concentrations of ROS in their own cells. Indeed, low ROS concentrations are known to be required for signalling, growth and development, while high concentrations are detrimental to the cell and can damage various macromolecules. The antioxidants include the low-molecular-weight compounds glutathione, ascorbate and tocopherol and the enzymes superoxide dismutases, catalases, ascorbate- or thiol-dependent peroxidases, glutathione reductases, dehydroascorbate reductases and monodehydroascorbate reductases (Rouhier *et al.*, 2008). These enzymes are involved in the removal of ROS either directly (superoxide dismutases, catalases, and ascorbate- or thiol-dependent peroxidases) or indirectly through the regeneration of the two major redox molecules in the cell, ascorbate and glutathione (glutathione reductases, dehydroascorbate reductases and monodehydroascorbate reductases). An interesting feature of the interplay between oxidants and antioxidants is that it occurs in all subcellular compartments including plastids and mitochondria, two sites of extensive ROS production (Navrot *et al.*, 2007). Of primary importance for the development of plant–microbe interactions are the ROS produced at the interface between the partners, that is, in the extracellular matrices, cell walls and more generally the apoplast compartment. NADPH oxidases, plasma membrane-situated proteins, are key players in this subcellular compartment for the generation of ROS species including superoxide ions and hydrogen peroxide.

'... the molecular limits between pathogenic and mutualistic associations are sometimes very narrow ...'

It has long been assumed that symbioses such as ectomycorrhizas, arbuscular mycorrhizas and rhizobial–leguminous interactions can be, in several aspects, beneficial to the plant partner (Jones *et al.*, 2007; Finlay, 2008). It has been shown that these interactions can contribute to increased plant resistance or tolerance to several biotic or abiotic constraints. The recent paper by Baltruschat *et al.* (2008) demonstrated that the root endophytic basidiomycete *Piriformospora indica* increases the tolerance of a salt-sensitive barley (*Hordeum vulgare*) cultivar to severe salt stress. This paper follows a previous article indicating that this fungus also improves plant resistance against root and leaf diseases (Waller *et al.*, 2005). Under these salt stress conditions, *P. indica*-colonized plants contained higher ascorbate concentrations in roots compared with noncolonized plants, while the ratio of ascorbate vs dehydroascorbate was not significantly altered and catalase, ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase and monodehydroascorbate reductase activities were increased. These modifications are consistent with the decrease of leaf lipid peroxidation observed in these experiments.

In this issue of *New Phytologist*, the paper by Xu *et al.* describes an unexpected beneficial aspect of plant–pathogen interactions. They used 10 monocot and dicot plant species (*Beta vulgaris*, *Capsicum annuum*, *Cucumis lanatus*, *Cucumis sativus*, *Solanum lycopersicum*, *Oryza sativa*, *Cucurbita pepo*, *Chenopodium amaranthicolor*, *Nicotiana benthamiana* and *Nicotiana tabacum*) and inoculated them with the specific RNA viruses CMV (Cucumber Mosaic Virus), BMV (Brome Mosaic Virus), TMV (Tobacco Mosaic Virus) and TRV (Tobacco Rattle Virus). The infected plants exhibited better tolerance and survival in response to drought and/or cold stress, suggesting that the viral infection induced a reaction that may be part of an elaborate mechanism used by plants to survive under various environmental challenges. It is likely that the presence of the viruses up-regulated a specific set of stress-related genes which allows the infected plant to survive for a longer period when subjected to additional abiotic stresses, which are also known to generate the production and accumulation of ROS (Apel & Hirt, 2004). The findings of this study are consistent with the improved thermal tolerance observed for the plant and fungal partners of a tripartite mutualistic interaction between the fungal endophyte *Curvularia protuberata* and the tropical grass *Dichantelium lanuginosum* only when a virus is present in the fungal isolates (Márquez *et al.*, 2007). In these cases, the contact with the
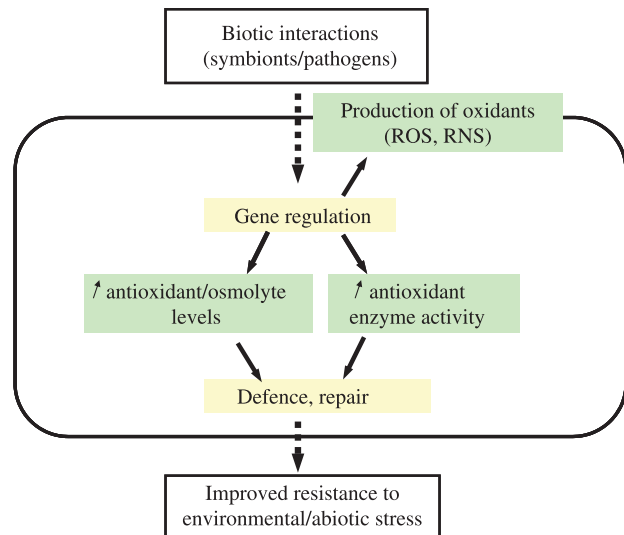
Fig. 1 Interplay between oxidizing and reducing pathways in biotic and abiotic interactions. The defence mechanisms induced during a biotic stress may in some cases enhance plant tolerance to abiotic stresses. The converse scenario, that is, that an abiotic stress would render the plant more tolerant to a pathogen attack, might also be true in some systems but is not represented here (see text). Reactive oxygen species (ROS), including superoxide ions, hydrogen peroxide and hydroxyl radicals, and reactive nitrogen species (RNS), such as nitric oxide and peroxynitrite, participate in the redox war occurring at the interaction frontier and are essential to both pathogenesis and symbiosis. The antioxidants and antioxidant enzymes exemplified here belong essentially to the ascorbate–glutathione cycle and to the peroxide scavenging systems (catalases and ascorbate peroxidases).

virus or pathogen induced molecular changes in the plant hosts which made them more tolerant to other stresses. Following these experiments, one wonders whether pathogens can also provide useful metabolites or enzymes that could be of benefit to their hosts. These studies demonstrate that the molecular limits between pathogenic and mutualistic associations are sometimes very narrow, as shown for the interaction between *Epichloë festucae* and *Lolium perenne*, where the inactivation of one gene results in a different life style (see above; Tanaka *et al.*, 2006).

Overall, these studies indicate that the increased plant tolerance to abiotic stresses (whether drought, salt or cold/thermal stress) recorded when plants are in contact with a microbe, either a pathogen or a mutualist, is in part correlated with an increase in antioxidant or osmolyte concentrations and/or in the activities of antioxidant enzymes, with ascorbate apparently playing a major role in the plant cells (Baltruschat *et al.*, 2008), as illustrated in Fig. 1. These observations may somehow be related to the systemic acquired resistance observed in some pathogenic interactions where healthy parts of the host plant become more resistant to a subsequent infection by either the same microbe or another one. As far as we know,

there is no molecular evidence for the involvement of the above-mentioned antioxidants in this process. Of course, in addition to ascorbate, several other compounds are also crucial and it is well known that glutathione and several hormones (abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA) and ethylene) are important players both in the abiotic stress response of plants and in plant–microbe interactions (Chamnongpol *et al.*, 1998; Asselbergh *et al.*, 2007; Vadassery *et al.*, 2008). Following the reports of Xu *et al.* and Baltruschat *et al.*, it will be of interest to determine the concentrations and qualities of antioxidants generated in various plant–microbe systems and to test whether this antioxidant generation results in improved stress protection for one or the other partner. Such data would allow further testing of the hypothesis that we have made in this commentary, i.e. that the biotic stress generates various antioxidants that help withstand an additional biotic stress.

Another important area of research would be to determine whether the converse is true; that is, do plants subjected to an abiotic stress become more resistant to other abiotic or biotic stresses? Although it is expected that a prolonged exposure to an abiotic stress would weaken plant defences and thus render the plants more susceptible to pathogen infection, there are a few contradictory examples. Bilgin *et al.* (2008) showed that ozone stress enhanced soybean (*Glycine max*) tolerance to a virus attack and Chamnongpol *et al.* (1998) demonstrated that a tobacco (*Nicotiana tabacum*) plant deficient for catalase, and thus more sensitive to photooxidative stress, exhibited enhanced tolerance against a pathogen attack. In their natural environment, plants may indeed be subjected simultaneously or sequentially to a combination of biotic or abiotic stresses. This might result in conflictive situations, because abiotic stress would promote an increase in antioxidant defences to scavenge the ROS produced, while the pathogen challenge would require a lowering of those defences for increased production of ROS, at least at the infection sites. In addition, it is worth mentioning that all oxidative stress conditions do not lead to identical patterns of response; although there are general oxidative stress response markers, additional unique and specific pathways are induced by specific stresses and specific ROS (Gadjev *et al.*, 2006, Laloi *et al.*, 2007).

In conclusion, the beneficial effect of viral infection for abiotic stress tolerance is still no more than a 'pis-aller' in terms of improving agricultural yields. Indeed, whilst the beneficial effect of viral infection can temporarily delay the negative effects of a given abiotic stress, it cannot protect indefinitely against them. Nevertheless, the papers discussed here provide an interesting molecular paradox that might help in the engineering of more stress-resistant plants to mitigate against the impacts of global climate change on agricultural and native plant communities via the enhancement of their redox defences, for example through the use of disarmed viral strains or fungal symbionts.

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Letters

Ectomycorrhizal fungi from Alaska and Pennsylvania: adaptation of mycelial respiratory response to temperature?

Despite the importance of ectomycorrhizal (ECM) fungi to both the carbon economy of their hosts (Smith & Read, 1997; Wallander *et al.*, 2004) and ecosystem respiration (Rygiewicz & Andersen, 1994; Bååth & Wallander, 2003; Hobbie & Hobbie, 2006; Heinemeyer *et al.*, 2007; Moyano *et al.*, 2008), little is known about the factors that control their rates of respiration. We have recently found that substantial variation exists among ECM fungal isolates in the ability of respiration to acclimate to temperature (Malcolm *et al.*, 2008). The potential for respiratory adaptation to temperature, an evolutionary response, has yet to be shown for ECM fungi. Therefore, in this study, we explored variation in respiration for ECM fungi in culture from two sites located at different latitudes.

Fungi are presumed to adapt to prevailing temperatures because species taken from different climates exhibit temperature ranges for optimal growth, or exhibit distinct minimum or maximum temperatures for survival that correspond to their climates of origin (Cooke & Whipps, 1993; Tibbett *et al.*, 1998; Robinson, 2001). The same is true for plants. With the presumption that temperature exerts a strong selective effect on respiration along environmental gradients (Reich *et al.*, 1996), studies utilizing elevational or latitudinal gradients have shown repeatedly that plant respiration rates at a given measurement temperature are frequently higher for ecotypes from northern latitudes or higher elevations than for those from southern latitudes or lower elevations (Sowell & Spomer, 1986; Mariko & Koizumi, 1993; Reich *et al.*, 1996).

Therefore, we hypothesized that cultures of ECM fungi from near Fairbanks, Alaska would have higher respiration rates at a given measurement temperature than those from near State College, Pennsylvania, which, if found, would be suggestive of adaptation to temperature, as has been found for plants along environmental gradients. We also determined whether isolates from contrasting sites differed in respiratory sensitivity to temperature (Q_{10}) in order to help predict whether the effects of future shifts in soil temperature on the respiration rates of ECM fungi depend on site.

Because different fungal lineages have different evolutionary histories, it may not be valid to contrast an isolate of one lineage from higher latitude with another isolate of a different lineage from lower latitude (Burt, 1989). Thus, while we were not able to collect isolates of the same species from contrasting sites, we did make comparisons using congeneric contrasts. Specifically, we collected ECM fungi from four genera and three families: Amanitaceae (*Amanita* spp.), Russulaceae (*Lactarius* spp.) and Boletaceae (*Leccinum* spp. and *Suillus* spp.), each from both Alaska (latitude 65°07′N, longitude 147°30′) and Pennsylvania (latitude 40°48′N, longitude 77°54′) (Table 1).

Fungi were cultured from field-collected sporocarps and maintained on a growth medium consisting of 19.5 g of potato dextrose agar (Difco; Becton, Dickinson & Co., Sparks, MD, USA), 7.5 g of Bacto Agar (Difco) and 0.375 g of NH_4Cl per liter of water. Fungal cultures were initially maintained in incubators set to temperatures reflective of those experienced by the fungi during the growing season at their site of origin: 17°C for Pennsylvanian isolates and 11°C

Table 1 Details of collection of eight isolates of ectomycorrhizal (ECM) fungi from four different genera and three different basidiomecete families: Amanitaceae (*Amanita* spp.), Russulaceae (*Lactarius* spp.) and Boletaceae (*Leccinum* spp. and *Suillus* spp.)

ECM fungal species	Isolate	Collected	Vegetation
Amanita muscaria var. *muscaria*	GL015	South Fairbanks, AK	*Betula papyrifera, Populus balsamifera, Picea glauca*
Amanita citrina	SC070	State College, PA	*Pinus resinosa*
Lactarius cf. *pubescens*	AK010	Chatanika, AK	*Betula papyrifera, Populus balsamifera*
Lactarius chrysorrheus	SC016	State College, PA	*Pinus resinosa*
Leccinum cf. *alaskanum*	GL005	South Fairbanks, AK	*Betula papyrifera, Populus balsamifera, Picea glauca*
Leccinum aurantiacum	SC014	State College, PA	*Pinus resinosa*
Suillus intermedius	BX007	State College, PA	*Pinus resinosa*
Suillus cf. *grevillei*	AK014	South Fairbanks, AK	*Picea mariana*

for Alaskan isolates (for more information about environmental temperatures, see Malcolm *et al.*, 2008).

Two isolates (one from Pennsylvania and one from Alaska) from each of the four genera were propagated using the growing medium described above in each of 10 100 × 20 mm Petri dishes, for a total of 80 dishes. Each isolate was initially grown at 11°C (Alaskan isolates) or 17°C (Pennsylvanian isolates) for 2–3 wk in order for each fungal colony to initiate new growth (*c.* 2–4 mm increase in diameter). After new growth had been established, half of the 10 dishes of each isolate were maintained in the initial incubator. The other half were shifted to the other incubator. Each of the replicate dishes was maintained for 1 wk in these incubators before respiration was assessed in order to allow for the potential for physiological acclimation to temperature (Malcolm *et al.*, 2008).

Steady-state respiration rates (CO_2 exchange rates) were determined at 11, 17, and 23°C for each of the 80 dishes using a custom-built gas exchange system (Malcolm *et al.*, 2008). In order to standardize respiration rates by fungal dry weight, fungal tissues were separated from agar by melting in test tubes held in boiling water immediately following the respiration measurements. The tissues were placed in a drying oven (60°C) until constant weight was achieved.

The proportional change in respiration rate over a 10°C interval (Q_{10}) was calculated by plotting the log-transformed respiration rates against measurement temperature, obtaining the slopes of the linear regression, and using the following equation (Atkin *et al.*, 2000):

$$Q_{10} = 10^{(10 \times \text{slope})}$$

Because respiration rates were obtained across a range of measurement temperatures on the same set of individual cultures, we used the Repeated Measures Proc Mixed ANOVA Procedure in SAS (version 9.1, 2002–2003; SAS Institute Inc., Cary, NC, USA) to analyze the respiration responses to temperature. We determined the effects of site, genus, measurement temperature and the interaction between site and measurement temperature on respiration rate when: isolates from Alaska were incubated at 11°C and isolates from
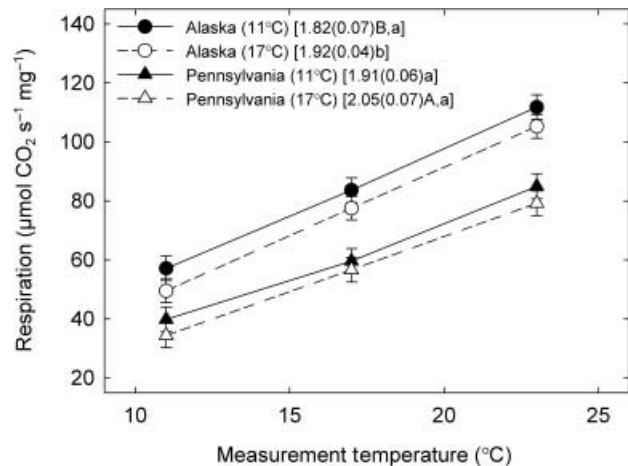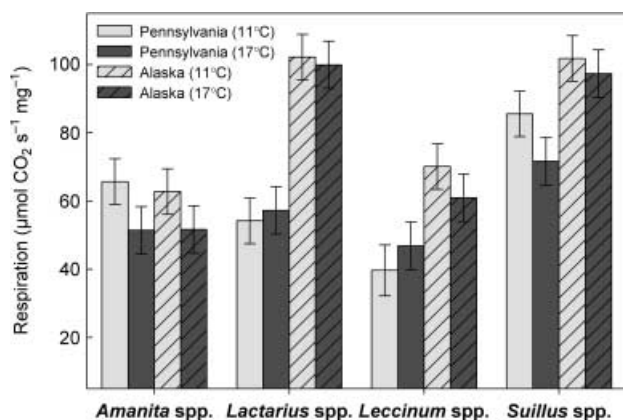

Fig. 1 Mean (± 1 SEM) of respiration at three measurement temperatures for ectomycorrhizal fungi originating from Alaska and Pennsylvania incubated at 11 and 17°C. Mean (SEM) Q_{10} values are displayed for each site of origin at each incubation temperature. Q_{10} values followed by different uppercase letters are significantly different for the comparison of fungi from Alaska and Pennsylvania incubated at 11°C and 17°C, respectively; and Q_{10} values followed by different lowercase letters are significantly different for the comparison of fungi from both sites incubated at a common temperature of 11 or 17°C. $n = 4$ genera.

Pennsylvania were incubated at 17°C; all isolates were incubated at a common temperature of 11°C; and all isolates were incubated at a common temperature of 17°C. To analyze Q_{10} we used the Proc GLM ANOVA Procedure in SAS with site and genus as main effects. Differences were analyzed in the same cases as above.

Irrespective of whether the isolates were incubated at temperatures reflective of their sites of origin (Alaskan isolates at 11°C and Pennsylvanian isolates at 17°C) or at common temperatures (either 11 or 17°C), the mean respiration rate was significantly higher at a given measurement temperature for isolates from Alaska than for isolates from Pennsylvania over the range 11–23°C (Fig. 1, Supporting information Table S1). The significant effect of site of origin was observed in all

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Fig. 2 Mean (± 1 SEM) of respiration, averaged over measurement temperatures of 11, 17 and 23°C, for each of four genera of fungi originating from Alaska and Pennsylvania. Fungi were incubated at 11 or 17°C. *n* = 5 Petri dishes for each combination of site × genus × treatment.

genera except for *Amanita* (Fig. 2). Furthermore, the average respiration rate of Alaskan isolates at a measurement temperature of 11°C (57.16 ± 4.17 µmol CO$_2$ s^{-1} mg^{-1}) was very close to that of Pennsylvanian isolates at a measurement temperature of 17°C (56.70 ± 4.17 µmol CO$_2$ s^{-1} mg^{-1}), irrespective of incubator temperature (Fig. 1).

These results are consistent with the hypothesis that the metabolism of ECM fungi can adapt to some factor related to site of origin. Because the two sites differ markedly in latitude, this factor may be temperature, but this is impossible to determine unequivocally because, in addition to temperature, vegetation type, soil chemistry and moisture may also differ between sites. Nevertheless, temperature differences along latitudinal gradients appear to create a strong selective pressure on respiration responses in plants (Reich *et al.*, 1996), so it is possible that adaptation to temperature contributed at least partly to the observed phenomenon in ECM fungi. The large number of studies performed on plant latitudinal and altitudinal gradients with results that are consistent with adaptation to temperature (Sowell & Spomer, 1986; Mariko & Koizumi, 1993; Reich *et al.*, 1996), as are ours, suggests that, if temperature is not the only factor to which organisms have adapted along such gradients, it may certainly be an important contributing factor.

The Q_{10} values reported for the fungi in this study, between 1.82 and 2.05 for measurement temperatures between 11 and 23°C (Fig. 1), were close to the often-assumed value of 2.0 for most biological systems (Cox *et al.*, 2000; Potter *et al.*, 2001; Atkin *et al.*, 2005). We have shown, however, that site may influence the sensitivity to temperature of ECM fungi. Overall, values of Q_{10} were significantly lower for isolates from Alaska than for isolates from Pennsylvania, except when the isolates were incubated at a common temperature of 11°C

(Fig. 1, Table S2). Thus, as global temperatures increase, proportional increases in respiration for a given temperature shift could be lower for the fungi at high latitude than for those at lower latitude. Few comparable data exist for ECM fungi, but in studies of plants, Sowell & Spomer (1986) and Mariko & Koizumi (1993) indicated that no significant variation in Q_{10} was seen among ecotypes from different elevations. Further, the sensitivity of the respiration response to temperature is similar to that of roots, mycorrhizal fungal hyphae, and soil microbes, as previously shown by Bååth & Wallander (2003) in microcosm.

We acknowledge that the respiration rates reported herein may not necessarily reflect absolute rates for fungi *in symbio* because of potential host effects on carbon supply. However, the respiration rates obtained in this study do agree with those obtained in previous studies of ECM fungi in culture (Taber & Taber, 1987; Souto *et al.*, 2000; Malcolm *et al.*, 2008), with those obtained *in symbio* (Rygiewicz & Andersen, 1994), and also with a field estimate calculated by Malcolm *et al.* (2008). Still, we urge modelers to use caution if utilizing data from this study, but to take note of the broader ecological pattern that we have shown.

In summary, our results suggest that the metabolisms of ECM fungi from two widely divergent sites from varying latitudes may be inherently different which could, in turn, have far-reaching implications for the carbon economy of the host plant and ecosystem respiration. For example, if the host does have less control over carbohydrate transfer to the fungus than the fungus itself, then for plant hosts from lower latitudes, fungal isolates from higher latitudes may be inappropriate symbionts because of their disproportionately high respiration costs. Moreover, because ECM fungi contribute significantly to total soil respiration (Heinemeyer *et al.*, 2007; Moyano *et al.*, 2008), adaptation to prevailing temperatures would tend to equalize their contribution to total soil respiration rates along temperature gradients. Finally, our use of congeneric contrasts from a variety of families suggests a general propensity for physiological adaptation among the Basidiomycota. Further studies that include more latitudes and/or more replication within latitudes, and studies that consider the respiration response of ECM fungi *in symbio*, appear to be warranted.

Acknowledgements

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Analysis of variance of respiration of ectomycorrhizal fungi incubated at temperatures reflective of the litter of their sites of origin during the growing season (11°C for Alaskan isolates and 17°C for Pennsylvanian isolates) or at common temperatures (either 11 or 17°C).

Table S2 Analysis of variance for the respiration Q_{10} value of four genera of fungi, each comprising two isolates, one from Alaska and one from Pennsylvania

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Meetings

Niche picking: the importance of being temporal

Environmental fluctuation, temporal dynamics and ecological processes symposium, Ecological Society of America (ESA) Annual Meeting, Milwaukee, WI, USA August, 2008

It is increasingly evident that adaptation to the frequencies, not simply to the extremes, of environmental fluctuation can regulate the abundances of populations and the structure and thus function of ecological communities. A recent symposium at the 2008 Ecological Society of American annual meeting in Milwaukee, entitled *Environmental fluctuation, temporal dynamics and ecological processes*, presented a range of studies demonstrating practical ways of approaching such temporal dynamics. The goal of this symposium was to confront the general theory of temporal dynamics with data and with more specific theoretical applications, highlighting a means of investigating this fundamental ecological process.

'The appearance of temporal dynamics in a diversity of communities suggests a ubiquitous presence, at least in the plant world'

Traditionally, a central aspect of species diversity is the differentiation of niches: species may co-exist over the long term when they possess sufficiently different requirements. Commonly invoked mechanisms of niche differentiation pinpoint species differences in a spatial or seasonal context, so that species co-exist because they complete essential parts of their life cycles in different parts of the habitat or at different times. Spatio-temporal dynamics allow the co-existence of species segregated into patches created by disturbance or edaphic variation.

By contrast, in temporal dynamics the population of established (reproductive) individuals is long-term stable over time; the competitive dynamic is played out by seedlings and juveniles, producing recruitment alternating between species over years, decades or even to the extent of a century or more in long-lived taxa. Unless explicitly taken into account, temporal niche dynamics can easily look like an absence of species interaction.

Temporal dynamics supported by noncatastrophic environmental fluctuation have been invoked as a regulatory agent in desert annual communities (Pake & Venable, 1996), Great Plains grasslands (Adler *et al.*, 2006), Mediterranean-type scrubland (Facelli *et al.*, 2006) and tropical forests (Kelly & Bowler, 2002), and involve a large proportion of the plant species within these communities (Kelly *et al.*, 2008). The appearance of temporal dynamics in such a diversity of communities suggests a ubiquitous presence, at least in the plant world. Whether or not temporal dynamics also apply directly to nonplant taxa, the role of plants as mediators between above-ground and below-ground ecological processes provides the potential for carry-on effects between, as well as within, trophic levels.

The theory of temporal niche dynamics was set out long ago, in a lottery model for fish species' co-existence (Chesson & Warner, 1981). The model specified environmental fluctuations affecting recruitment differently for different species as the starting point for dynamic long-term co-existence in which the populations continually fluctuate about a long-term mean. Given this background, the first necessity is 'storage' of reproductive capacity to allow recovery from unsuccessful seasons: long-term reproductive viability or persistent propagules. The second necessity is that species recruit at different times (or at different rates which also fluctuate) as a result of environmental differences; for example, species 1 recruits strongly when times are good for species 1, and species 2 recruits strongly when times are good for species 2.

To promote or permit co-existence, further criteria need to be satisfied. During good times a species at high density (considerably higher than the long-term mean) must not benefit strongly because the tendency must be to fall back towards the long-term mean. The per capita recruitment must therefore be suppressed at high density and this is accomplished through competition; mostly intra-specific. At low density (much lower than the long-term average) a species must be able to recover strongly and the population density grow towards the mean. The suppressing effect of competition needs to be minimal in good times at low density. Competition should thus be strongest under good conditions and weakest under poor conditions – both stabilizing influences. This is called covariance between competition and the environment [cov(EC)], which, to be effective, increases with increasing population density.

As a determinant of the co-existence of similar species, temporal dynamics are likely to be a major factor in community diversity and function. Nonetheless, temporal dynamics have yet to be well integrated into the larger ecological canon. Among recent ecology texts, only one treats temporal niche dynamics in any detail (Gurevitch *et al.*, 2006); out of a number of recent reviews on co-existence mechanisms, only one directly addresses temporal niche processes (Chesson, 2000). Anecdotal evidence from the general ecological community suggests that this is because temporal dynamics are seen to be difficult to understand and require long-term investment – longer term than is available within the structure of most careers in ecology. As shown at this symposium, this need not be the case.

Demonstrating the effects of temporal dynamics

It is self-evident that in many cases, direct observation of temporal dynamics is a long-term endeavour. However, community ecology has a long and ongoing history of tackling large-scale and long-term processes through demonstrating instead the outcomes or effects of those processes, and several presentations focused on determining such effects for temporal dynamics. After a brief review of general theory behind the temporal storage effect, Peter Chesson (University of Arizona, USA) described a manipulative experiment geared to show the effects on herbaceous perennials of the storage effect over a relatively short term. Chesson and associates had reasoned that recruitment in a monoculture where competition is necessarily wholly intraspecific, and thereby stonger, should be more closely regulated and less variable in response to the same environmental conditions than recruitment in a polyculture (Chesson, 2008). In an Australian herbaceous community, Chesson and associates created monocultures of a native perennial *Lagenifera* species to compare *Lagenifera* recruitment over time under this treatment with simultaneous recruitment in natural polycultures of *Lagenifera* and *Poa*, finding support for their expectation in two experiments over separate time sequences of 14 months and 26 months.

In the same vein, Colleen Kelly (University of Oxford, UK) and Michael Bowler (University of Oxford, UK) used static data to show that the distribution of relative abundance within closely related species in a Mexican dry forest is best explained by pairwise focused competition within temporal dynamics. With this result, they were thereby able to tie together recent work on fractional abundance and an independent signal for storage dynamics within the same community (Kelly & Bowler, 2005; Kelly *et al.*, 2008). That distribution is not explained by community-wide neutrality, or by two-species interchangeability or competitive exclusion. Yet, the species abundance distributions for the whole community and for species that are members of congeneric pairs are both lognormal, an unplanned result with implications for the current on debate on niche vs neutral processes regarding community organization (Leibold, 2008).

Investigating the necessary conditions for temporal storage dynamics

Another approach to investigate temporal dynamics is to establish whether the necessary conditions for their action are present, and whether those conditions are strong enough to have an effect on community dynamics. Peter Adler (Utah State University, USA) and colleagues used a heritage data set – post dustbowl grassland censuses across the US heartland – to search for conditions necessary for co-existence through the action of temporal dynamics. For Kansas prairie grasses they found the presence of the key conditions (Adler *et al.*, 2006), but little evidence for environment–competition covariance, in an Idaho sagebrush community. They investigated whether the storage effect in the Kansas grasslands is sufficiently strong to permit co-existence, but the results were not conclusive.

Temporal dynamics may also be induced by biotic factors, which in turn are likely to be regulated by environmental conditions. Earlier work by Michael Hanley (University of Plymouth, UK) showed slug herbivory by seedlings on the UK's Salisbury Plain to have significant long-lasting effects on the cover of mature herbaceous plants (Hanley *et al.*, 1996 and subsequent data), with great variation from year to year. More recently, Hanley and Rebecca Sykes (University of Plymouth, UK) found experimental support for the role of differential sensitivity (DS) temporal dynamics by demonstrating that slug grazing on seedlings is sufficient to reverse the relative cover, at maturity, of congeneric pairs, with the more palatable, faster-growing species achieving more cover when grazing is absent or low and the less-palatable species gaining the upper hand when grazing pressure is higher.

Direct observation

Norma Fowler (University of Texas, USA) and Craig Pease (Vermont Law School, USA) did not set out to document temporal dynamics, but nonetheless did so through an ingenious approach that was as interesting for its novelty as its results. They used a simple model of population dynamics in studying 16 yr of data on eight species in a Texas grass-dominated herbaceous community. Year by year they solved the model equations with a separate carrying capacity, K, for each species and each year. They found that the population density lagged behind the carrying capacity K, growing after an increase in K and being too high to sustain after a drop. The lags and overshoots were not synchronous, and these results indicate an important role for temporal niche separation in the community structure of these arid grasslands.

Focused questions

Akiko Satake (Hokkaido University, Japan) and colleagues used theory to investigate the conditions under which pollination needs might drive the temporally varying patterns of mast

flowering and fruiting. They showed that the probability of plant species which share pollinators enhancing synchronized flowering and reproduction increased when the cost of producing a single flower increases simultaneously with a decrease of pollination rate with lower flower intensity (Satake & Iwasa, 2000, 2002; Iwasa & Satake, 2004). The model offers a promising approach to the ubiquitous phenomenon of intermasting intervals. It also offers another instance where interactions between tropic levels – and biodiversity – may mediate or be mediated by temporal fluctuations in the environment.

Conclusion

The essential message of this symposium, summed up by Gordon Fox (University of South Florida, USA), was that variability over time, as much as variability in space, allows species to co-exist, and the effect of temporal fluctuation appears to be ubiquitous. The studies presented and discussed above exemplify a growing body of work demonstrating this observation.

Although evidence is accumulating, temporal niche dynamics are still little studied, and this should not and need not be. Revelations of temporal dynamics are not restricted only to those already initiated into its mysteries. Some of the strong evidence here for temporal niche dynamics was 'found', rather than the result of efforts directed at testing the theory. While the signals of temporal dynamics may entail studies carried out over a substantial number of years (N. Fowler and C. Pease; detailed in the section entitled 'Direct observation') or long-term data sets (Adler *et al.*, 2006), they may also be found in static data (Kelly & Bowler, 2002) or in studies of only a few years (Chesson, 2008; M. Hanley and R. Sykes, detailed in the section entitled 'Investigating the necessary conditions for temporal storage dynamics) or in the targeting of specific theoretical questions (Satake & Iwasa, 2000, 2002).

Acknowledgements

I gratefully acknowledge the assistance of the speakers in preparing this report (all errors are, of course, my responsibility). I thank Chamela Biological Station, Jalisco, México, for its hospitality.

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The long hard road to a completed *Glomus intraradices* genome

Glomus Genome Consortium (GGC) Symposium, Nancy, France, September, 2008

With the public release of the *Populus* genome (Tuskan *et al.*, 2006), the United States Department of Energy's Joint Genome Institute (JGI) embarked on an effort to create a community-wide genomics resource for bacterial and fungal associates of *Populus* (Martin *et al.*, 2004). Included in the list of species were several *Populus* endophytes (*Burkholderia cepacia, Pseudomonas putida, Enterobacter* spp., *Serratia proteamaculans, Stenotrophomonas maltophilia*; http://genome.jgi-psf.org/draft_microbes/), *Laccaria bicolor* (Martin *et al.*, 2008),

Melampsora laraci-populina (http://www.jgi.doe.gov/sequencing/why/3088.html) and *Glomus intraradices* (http://www.jgi.doe.gov/sequencing/DOEmicrobes2004.html). Ideally, the development of genomic tools for these organisms will facilitate the study of *Populus* and of its microbial associates in experimental and natural environments using whole-genome microarrays, models of predicted metabolite and protein interactions, cross-species promoter analyses and molecular surveys of community diversity. Together these resources will provide the possibility to take a holistic approach in understanding how symbionts and pathogens interact with the host tree in contrasting environments.

The production of a completely annotated and assembled *G. intraradices* genome has proven to be an especially arduous challenge and, after 4 yr of effort, it is not yet at hand. In this context, a workshop was held by the *Glomus* Genome Consortium (GGC) on September 16–17, 2008, in Nancy, France, to review the progress that has been made to date on the *Glomus* genome.

'... sequencing of G. intraradices will have a tremendous impact on the scientific community as it will give first access to so far intractable information about processes driving the biology and life cycle of AM fungal symbionts'

At the root of the Mycota Kingdom

The arbuscular mycorrhizal (AM) symbiosis between fungi in the Glomeromycota and plants involves over two-thirds of all terrestrial plant species, and is of great ecological significance (Fitter *et al.*, 2000; Rosendahl, 2008). There are around 150 described species in the Glomeromycota, and about 200 000 plant species are involved in the symbiosis. The key process in the symbiosis is the acquisition of poorly mobile orthophosphate ions from soil by the fungi, greatly enhancing plant phosphorus (P) uptake (Smith & Read, 2008). Colonization of plants by AM fungi results in a 5–20% net increase in photosynthesis (Smith & Read, 2008). Thus, AM fungi make a very large, if poorly understood, contribution to the global carbon cycling budget of ecosystems. From an evolutionary standpoint, AM fungi are unique obligate symbionts with multinuclear coenocytic hyphae that transport organelles and nutrients over long distances. The regulation of gene expression in such a system with multiple nuclei migrating long distances is completely unexplored. Furthermore, the

concept of an individual is unclear because nuclei within a single AM fungus appear to be genetically different in some species. Although this has not been directly shown in *G. intraradices*, it raises substantial questions about the natural selection and population genetics of these highly unusual organisms (Kuhn *et al.*, 2001; Hijri & Sanders, 2005). There is no known sexual cycle in AM fungi, although anastomosis and nuclear movement between hyphae has been described (Giovannetti *et al.*, 2001).

Several factors have led to *G. intraradices* being chosen for the first genome sequencing of an AM fungus. It is a widespread fungal species in that it is present in different ecosystems throughout the world, including temperate and tropical locations (Smith & Read, 2008). As a symbiont, *G. intraradices* is highly effective in mobilizing, taking up and transferring mineral nutrients other than inorganic P, including nitrogen, from soils to plants (Govindarajulu *et al.*, 2005), and it readily colonizes many plant species, including agriculturally important species such as wheat, alfalfa, rice, as well as model plants such as *Medicago truncatula*, *Lotus japonicum* and *Populus trichocarpa*. *Glomus intraradices* is one of the most commonly studied AM fungi as it colonizes host plants rapidly. It is the prime ingredient in several commercial inocula. Moreover, it can be grown *in vitro* in dual culture with transformed carrot roots and it is the only species whose spores are available commercially in pure form in large quantities. Genome sequencing of *G. intraradices* will have a tremendous impact on the scientific community as it will give the first access to so far intractable information about processes driving the biology and life cycle of AM fungal symbionts.

Genome sequencing

The first insight into the global organization of the *G. intraradices* genome was in 2004 with the publication of an estimated genome size (Hijri & Sanders, 2004), based on flow cytometry and re-association kinetics, of 14–16.5 Mb per nucleus. In addition, 600 kbp of random genomic survey sequencing (GSS), spread among 680 sequences, were made available in GenBank. Subsequently, nearly 3000 expressed sequence tags (ESTs) from *G. intraradices* have been deposited in GenBank, along with nearly 1500 ESTs from other *Glomus* species. Over the last 2 yr, the JGI has achieved much more extensive whole-shotgun sequencing (WGS) of the *G. intraradices* DAOM 197198 isolate. About 80 Mb of WGS (i.e. five times more than the expected genome size) have been sequenced using the Sanger and 454 GS-20 pyrosequencing technologies. In addition, the sequences of 29 fosmids (~1.0 Mb) from the DAOM 197198 isolate have been finished by Jane Grimwood and her colleagues at the Stanford Human Genome Sequence Center (CA, USA). As expected for AM fungi (Hosny *et al.*, 1997), the genome of *G. intraradices* has a very low G+C percentage (~30%). At the GGC workshop, Harris Shapiro (JGI, CA, USA) presented

the assemblies obtained using these WGS data sets. He used three types of analysis to estimate the effective sequence depth of the existing WGS data set: (1) coverage of the available subcloned fosmid sequences by the WGS data, (2) the fraction of ESTs not covered by the WGS data, and (3) depths of contigs from Newbler assemblies of the WGS data. Poisson curve-fits of the depth of coverage of each contig base in the assembly ranged from 1.73 ± 0.12 to 2.38 ± 0.22. However, as the assemblies are still at a fairly low depth, it is not possible to derive a precise depth estimate from these data alone. Possible technical, informatic and biological reasons that may underlie this were discussed at length at the GGC workshop, without a clear answer emerging. One favoured possibility is that WGS assembly is hindered by the occurrence of multiple copies of many (perhaps most or all) nuclear genes, somewhat diverged in sequence – for which genomic and EST sequences provide evidence. A high level of polymorphism is evident within *G. intraradices*, so that some regions of the genome appear as different assemblies ('haplotypes') within the set of scaffolds. The presence of alternative haplotypes leads to a larger effective genome space. The number of gene/genome copies is still unknown, but this raises interesting questions about functional vs pseudogene copies, and about possibly different expression patterns for different alleles. Most software packages that are used to assemble WGS data cannot effectively segregate reads between two (even less between several) alleles. To complicate matters further, the genome is rich in short repeated sequences that increase the chance of misassemblies and are easily confused with alleles. Sequence contigs that end in repeated sequences can usually be joined with the help of higher-order information from the genome's organization. Unfortunately, the lack of a sexual cycle in *G. intraradices* has precluded the production of a meiotic genetic map.

Dr Mohamed Hijri's group (IRBV, Université de Montréal, Canada), in collaboration with Génome Québec, has carried out two 454 Genome Sequencer runs (containing a total of 113 Mb in 503 697 reads) on genomic DNA from the DAOM 197198 isolate. Finally, the University of York, UK (Peter Young *et al.*), has carried out three half 454 Genome Sequencer runs on another *G. intraradices* isolate (494) and reported on their findings. They generated 152 Mb of sequence in 687 751 reads that have been assembled using the most recent Roche DeNovo assembler, generating an assembly of 25 Mb; the largest contig is 32 kbp (a mitochondrial contig).

As we write, the novel 454 pyrosequencing data have been merged to the JGI sequences (i.e. 345 Mbp of WGS in total) and the most recent assembly is 52.5 Mb in 163 968 contigs. The depth has edged up from two times, to just more than three times the genome coverage. The lack of effective sequence depth is probably the result of a genome space that is much larger than the amount of DNA per nucleus. This space is now estimated to be > 150 Mb. Single nucleotide polymorphism variation among the genomic reads has been observed in many contigs, confirming the high polymorphism of the

G. intraradices genome. The JGI is planning to sequence 96 more fosmids by shearing, end tagging and pooling subclone DNA for a 454 FLX Titanium run. The INRA GlomusDB database (http://mycor.nancy.inra.fr/IMGC/GlomusGenome/) allows the GGC members access to the current sequence data.

Mitochondrial genome

The JGI, Université de Montréal and the University of York 454 data sets have allowed good assembly of the *G. intraradices* mitochondrial genome, which shows much lower levels of sequence polymorphisms than either EST or genomic sequences. Annotation of the mitochondrial genome (70 608 bp) of the *G. intraradices* 494 isolate was presented at the GGC workshop by P. Young. This genome contains a standard set of fungal mitochondrial proteins and RNA genes, introns and LAGLIDADG homing endonuclease genes.

EST sequencing and clustering

Diederik Van Tuinen (INRA-Dijon, France), Philipp Franken (Institute of Vegetable and Ornamental Crops-Grossbeeren), Helge Küster (Leibniz Universität Hannover, Germany), Yair Shachar-Hill (Michigan State University, USA) and Francis Martin (INRA-Nancy, France) updated the GGC on the current status of EST sequencing and EST databases. *Glomus intraradices* ESTs are derived from polymerase chain reaction-amplified or normalized cDNA libraries of spores germinated in the absence or presence of strigolactones/flavonoids and extraradical symbiotic mycelium (ERM). The EST data set contains 83 539 clusters (or tentative consensus sequences) assembled from 318 945 Sanger and 454 ESTs generated by JGI, New Mexico State University, Michigan State University, INRA/Toulouse University and the German MolMyk programme. The high number of EST clusters results from high EST sequence polymorphism. While functions of 7750 transcript clusters may be inferred from sequence homology to genes in other organisms, most of the predicted genes still have no known function and genes so far unique to *G. intraradices* represent a very large proportion of the total predicted transcript set. This is a surprisingly high percentage that probably reflects both the symbiotic lifestyle and the evolutionary distance between *G. intraradices* and other fungal genomes sequenced to date.

Sequences of raw ESTs and EST clusters, and their automated annotation are stored at both the INRA GlomusDB database and the MolMyk SAMS database (http://www.cebitec.uni-bielefeld.de/legume/molmyk.html). Additional EST sequencing on laser-dissected intraradical mycelium (IRM) and isolated arbusculated cells from colonized rice and *Medicago* roots using the Illumina-Solexa and 454 GS FLX Titanium is underway. A NimbleGen array is under construction using transcript sequences.

Unwrapping the *Glomus* genome: a formidable task ahead

Several key questions remain unanswered. Is polymorphism overall low enough to eventually get a good WGS assembly? Are certain regions of the genome more subject to polymorphism? Can we make an assembly with the rest? When looking at expression, are we seeing the real picture or are there many other variants of a given gene that are not being expressed? By mid-2009, the mycorrhizal research community may have access to more genomic information that will prove valuable for investigations of the plasticity and evolution of *G. intraradices*.

The JGI and the GGC are pleased to announce that the initial genome assembly and EST sequencing data (as of December 2008), and their automated and manual annotations, will be made available to the scientific community in January 2009 through the INRA GlomusDB database.

Acknowledgements

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Key words: arbuscular mycorrhizal fungi, clustering, expressed sequence tag (EST), genome, *Glomus intraradices*, Joint Genome Institute (JGI), sequencing, symbiosis.