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Variable effect of arthropod-induced galls on photochemistry of photosynthesis, oxidative enzyme activity and ethylene production in tree leaf tissues

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Abstract

The aim of the present experiments was to determine the effect of arthropod-induced galls on photochemistry of photosynthesis, defense enzyme activity and ethylene production in host plant tissues. In total, 10 arthropod gall-inducer–host plant combinations, including seven tree species infected with five species of eriophyid mites, four aphid species and one sawfly species were analyzed. The presence of gall tissues differentially affected photochemistry of photosynthesis in non-galled adjacent leaf tissues. Localization of leaf vein pocket galls induced by *Colopha compressa* on leaves of *Ulmus laevis* resulted in photoinhibition of photosynthesis in parallel with decreased electron transport rate and increased non-photochemical quenching. The degree of infestation with eriophyid mite *Eriophyes padi* was associated with decrease in photochemical efficiency of photosystem II and concomitant increase in non-photochemical quenching in leaves of *Prunus padus*. Both peroxidase and polyphenol oxidase activity increased with increasing number of sawfly *Pontania vesicator* galls per leaf of *Salix fragilis*. Similarly, peroxidase activity in leaves of *Acer saccharinum* increased along with increasing level of infestation with *Vasates quadripes*. The presence of aphid *Pemphigus spirothecae*-induced petiole spiral galls resulted in significant decrease of both enzyme activities in leaf blades of *Populus nigra* with no changes in leaf petioles. Gall formation resulted in significant increase in ethylene production from leaf blade tissues of *Populus nigra*. The presence of leaf vein pocket galls on leaves of *Ulmus laevis* resulted in more than two-fold increase of ethylene production rate in affected leaf tissues. Increased rate of ethylene production was found also in other gall forming arthropod-host plant combinations. The variable effect of gall formation on host plant photochemistry of photosynthesis and oxidative enzyme activity might be explained by specific gall-inducer–related signals at the site of activity, in combination with specific endogenous plant signals involving ethylene.

Key words: chlorophyll *a* fluorescence; ethylene; arthropod galls; peroxidase; polyphenol oxidase; trees.
Abbreviations: ETR, electron transfer rate; F_V/F_M, maximum efficiency of photosynthesis; NPQ, non-photochemical quenching; ROS, reactive oxygen species.

Introduction

Herbivores affect plant growth and performance by both physical removal of plant tissues and by indirect impact on physiological processes. Among indirect herbivore effects, those on photosynthesis might have the most significant impact on plant growth and performance. It is recognized that the magnitude of herbivore effects on photosynthesis is highly variable depending mostly on both the type of damage during feeding and on specificity and intensity of plant defense responses (Nabity et al. 2009). While defoliation usually does not alter or even increases photosynthesis in the remaining leaves, due to the compensation mechanisms, phloem feeding can result in inhibition of photosynthesis (Zvereva et al. 2010).

Gall-inducing arthropods do not remove leaf tissue, but form specialized feeding and sheltering structures that act as sinks, in a way similar to free-living aphids and other cell-content feeders. Therefore, gall-inducers can be considered as phloem parasites (Larson, Whitham 1997). It is widely believed that insects completely control redirection of growth and physiology of attacked plant organs to their own benefit (Shorthouse et al. 2005). However, a more realistic scenario includes mutual interaction between a gall-inducer and adjacent plant tissues both during gall establishment as well as within later phases of development, because it can be expected that during gall functioning arthropods continuously produce multitude of physical and chemical signals which are perceived by plant and induce changes of negative or positive consequences for plant physiology (Raman 2007).

Previous studies showed that an interaction between gall tissues and non-galled plant parts during neoplastic development and further plant–gall-inducer coexistence result in changes in plant adaptive responses (Gailite et al. 2005a). Manipulation of indirect plant defenses by galling insects has been recently explored (Tooker, De Moraes 2008). In general, volatile responses induced by chewing

herbivores were seen to effectively suppressed by a gall-inducer. In respect to direct defenses, these are supposed to be inhibited only in the near vicinity to nutritive tissues protecting a gall-inducer from intoxication (Aldea et al. 2006). In addition, downregulation of host-plant defense responses by gall-inducing sawflies has been described (Nyman, Julkunen-Tiitii 2000).

Decrease in oxidative enzyme activity in gall tissues is a general phenomenon (Gailite et al. 2005a). In contrast, high level of defense responses in other tissues of galled leaves could have possible benefit for a gall-inducer due to a better protection of the particular leaf against other herbivores or pathogens. If a sepcific continuously produced chemical signal(s) from gall tissue controls expression of defense-related genes, it might be expected that the response would be stronger in the case of multiple galls per leaf.

Ethylene is an endogenous signal molecule necessary for control of numerous herbivore-related defenses (von Dahl, Baldwin 2007). Ethylene is required for increased activity of peroxidase and polyphenol oxidase in bean plants due to regurgitant treatment at the level of appropriate gene expression and protein synthesis (Steinite et al. 2004). No study so far has described changes in ethylene production in arthropod-induced plant galls. Endogenous jasmonate is another well-known inducer of plant defense responses, including those against herbivores (Baldwin 1998). On the other hand, jasmonate inhibits photosynthesis-related gene expression (Reinbothe et al. 1993).

While several papers have described the effect of gall formation on certain aspects of photosynthesis (Fay et al. 1993; Larson 1998; Dorchin et al. 2006; de Oliveira et al. 2010; Fernandes et al. 2010), no complex studies involving different types of gall-inducer–host plant combinations on photosynthetic performance have been performed so far. The aim of the present study was to determine the effect of gall tissues on chlorophyll fluorescence characteristics in adjacent non-galled leaf tissues. Ethylene production was analyzed as a possible indication of defense activation. More specifically, it was hypothesized that multiple galls per leaf result in production of a stronger sub-systemic chemical signal and, consequently, more pronounced changes in putative defense responses than in the case of a single gall.

Materials and methods

Plant material

In total 10 arthropod gall-inducer–host plant combinations were analyzed in the present study (Table 1). Seven tree species, infested with five species of eriophyid mites, four aphid species, and one sawfly species were located in the National Botanic Garden of Latvia, Salaspils, Latvia. Material for analysis was collected in July during 2005 – 2009. Leaves with fully developed gall structures as well as control leaves with no visible signs of injury were used for analysis.

Measurement of chlorophyll a fluorescence

Chlorophyll *a* fluorescence was analyzed by two alternative methods – pulse amplitude modulated (PAM 2010, H. Walz, Effeltrich, Germany) and continuous measurement (Handy PEA, Hansatech, Kings Lynn, Norfolk, UK) according to manufacturer's instructions as described previously (Samsone et al. 2009; Andersone et al. 2011). Three to five independent fluorescence measurements using five appropriate leaves were performed for every data point.

Protein extraction and measurement of enzyme activity

Protein extraction and measurement of enzyme activity was performed as described previously (Andersone, Ievinsh 2002). Briefly, plant tissues were ground in liquid nitrogen and extracted with 25 mM HEPES buffer (pH 7.2). After centrifugation at 15 000 g_n, the supernatant was used for spectrophotometric measurement of protein content, peroxidase and polyphenol oxidase activity. Guiacol plus hydrogen peroxide and pyrocatechol were used as the respective substrates for the enzymatic assays.

Table 1. Host plant–gall-forming arthropod combinations and physiological aspects analyzed in the present study. fluor, chlorophyll *a* fluorescence; eth, ethylene production; def, defense-related enzyme activity

Host species	Gall-forming arthropod			
	Species	Order: Family	Gall type	Aspects analyzed
Acer saccharinum L.	*Vasates quadripes* Shimer.	Acarina: Eriophyidae	eriophyid mite galls	fluor, def
Populus nigra L.	*Pemphigus spirothecae* Pass.	Homoptera: Aphididae	petiole spiral galls	eth, def
Prunus padus L.	*Eriophyes padi* Nal.	Acarina: Eriophyidae	eriophyid mite galls	fluor, eth
Salix fragilis L.	*Eriophyes tetanotrix* Nal.	Acarina: Eriophyidae	eriophyid mite galls	fluor, def
Salix fragilis L.	*Pontania vesicator* Br.	Hymenoptera: Tenthredinidae	two-sided leaf galls	fluor, eth, def
Tilia platyphyllos Scop.	*Eriophyes tiliae* Pgst.	Acarina: Eriophyidae	eriophyid mite galls	fluor, eth, def
Ulmus glabra Huds.	*Eriosoma ulmi* L.	Homoptera: Aphididae	leaf-roll galls	fluor, eth
Ulmus glabra Huds.	*Tetraneura ulmi* L.	Homoptera: Aphididae	leaf pocket galls	fluor, eth
Ulmus laevis L.	*Aceria brevipunctata* Nal.	Acarina: Eriophyidae	eriophyid mite galls	eth
Ulmus laevis L.	*Colopha compressa* Koch.	Homoptera: Aphididae	leaf vein pocket galls	fluor, eth

Analysis of ethylene production

For ethylene analysis, intact control and galled leaves or detached appropriate tissues were incubated for 1 h in 4 mL borosilicate glass bottles, closed with teflon-coated silicone caps. Concentration of ethylene in gas samples was analyzed by a gas chromatograph Chrom 5 with a column filled with activated Al_2O_3 and flame ionization detector. Helium was used as a carrier gas. The amount of ethylene was calculated according to a standard curve. The tissue mass was measured after ethylene analysis both with and without galls. The data were expressed as rate of ethylene production per gram of leaf tissue. Five independent ethylene measurements using separate tissue samples for every data point were made.

Statistical analysis

Significance of differences between means were determined by the Tukey-Kramer test at the $\alpha = 0.05$ level.

Results

Photochemistry of photosynthesis

In control leaves of *Ulmus glabra* there was a characteristic gradient of chlorophyll *a* fluorescence parameters along a leaf, suggesting for differences in photochemistry of photosynthesis in different leaf parts. Maximum efficiency of photosynthesis (F_V/F_M) had a tendency to increase from the leaf base towards the middle part, decreasing again towards the leaf tip (Fig. 1A). Relative electron transfer rate of photosystem II (ETR) showed only a minor tendency to decrease from the base towards the tip of the leaf (Fig. 1B). However, non-photochemical quenching (NPQ) significantly increased along the leaf (Fig. 1C).

In leaves infested with the gall-inducing aphid *Tetraneuma ulmi*, on the distal part of the leaf, the character of distribution as well as the absolute values of the studied parameters were similar to those in the control leaves (Fig.

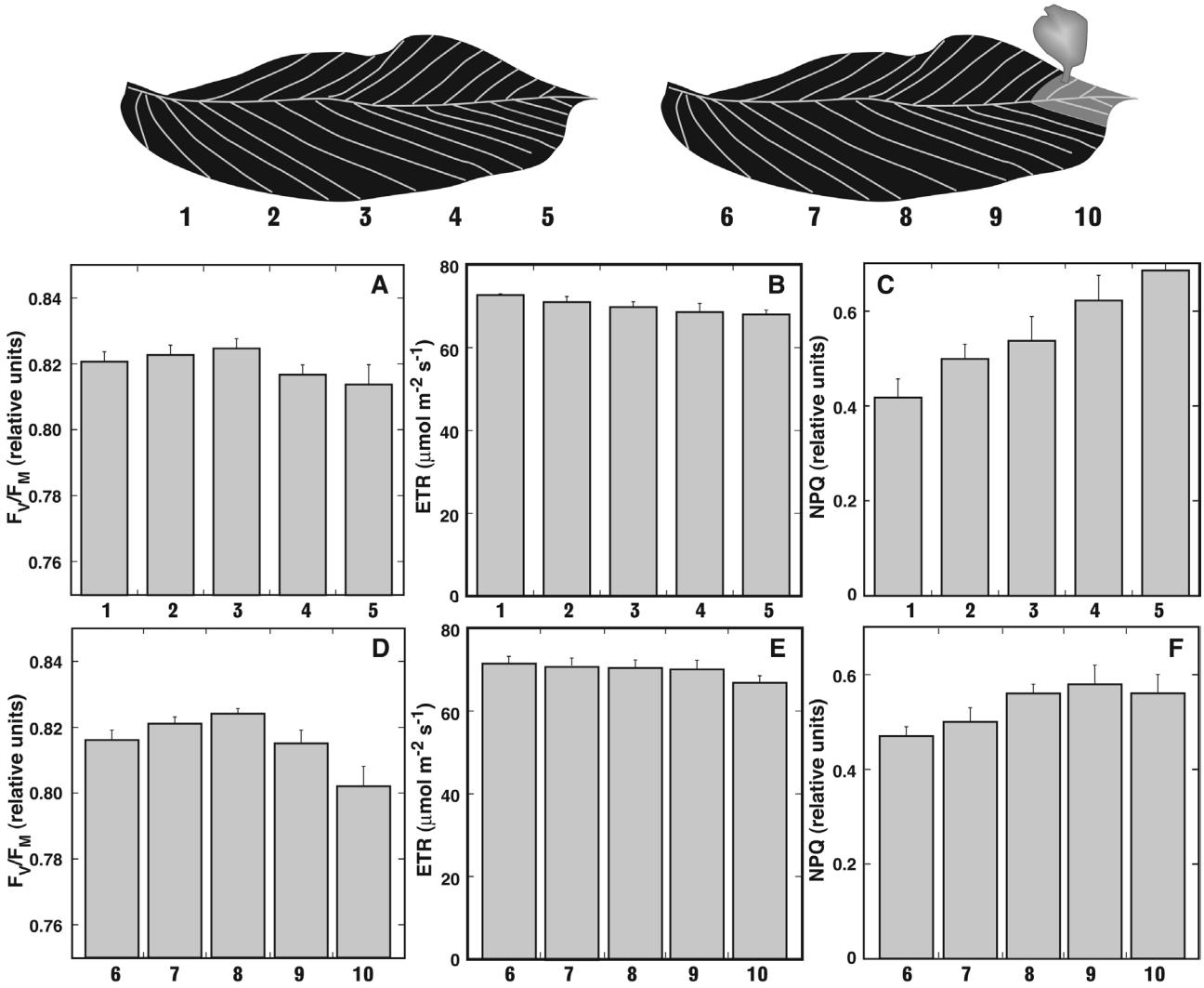

Fig. 1. Gradient of chlorophyll *a* fluorescence characteristics along a control leaf of *Ulmus glabra* (A, B, C) and a leaf infested with a gall-inducer *Tetraneuma ulmi* (D, E, F). Data are means ± SE from five leaves with five independent measurements per data point.

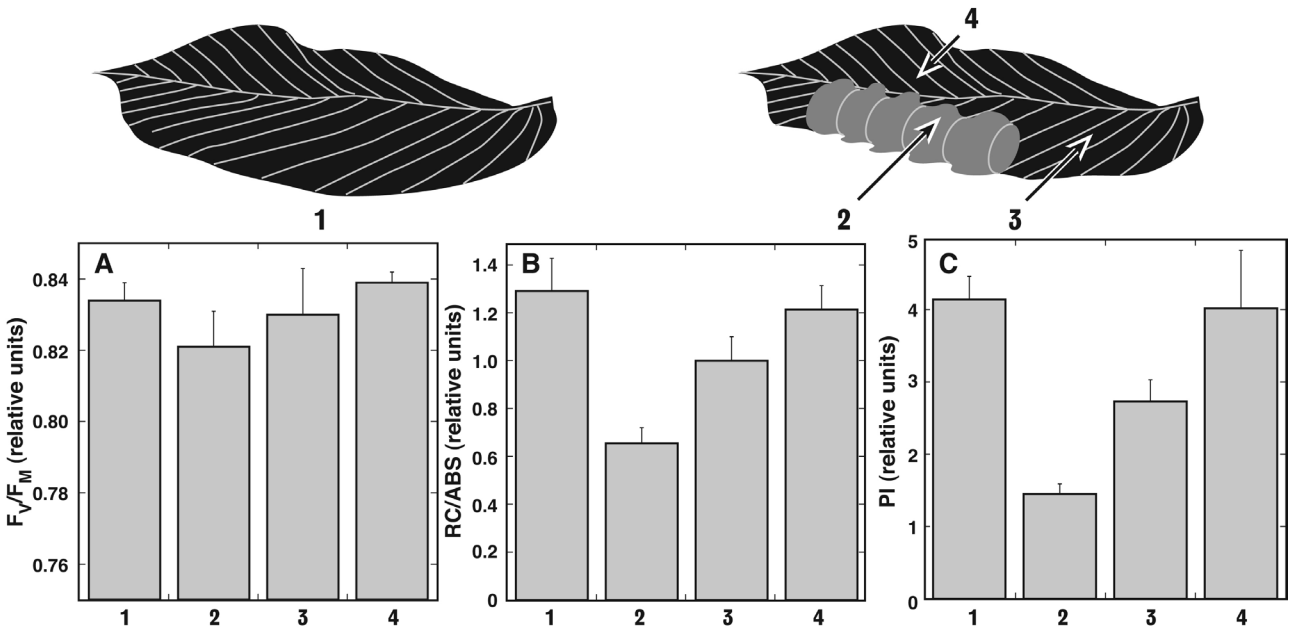

Fig. 2. Effect of infestation with *Eriosoma ulmi* on chlorophyll *a* fluorescence characteristics in leaves of *Ulmus glabra*. Data are means ± SE from five leaves with three independent measurements per data point.

1D - F), with the exception of significant decrease of F_V/F_M in the distal part of the leaf where gall was localized (Fig. 1D).

In leaves of *Ulmus glabra*, galled with the leaf-roll inducing aphid *Eriosoma ulmi*, photochemical efficiency of photosystem II significantly decreased in gall tissues as well as in the near vicinity to them (Fig. 2B, C). However, maximum efficiency of photosynthesis (F_V/F_M) was only nonsignificantly reduced, indicating no clear damage to photosystem II (Fig. 2A). In contrast, localization of *Colopha compressa* leaf vein pocket galls on leaves of *Ulmus laevis* resulted in significant damage to photosystem II in gall-affected leaf tissues, as suggested by a decline in F_V/F_M below 0.78 (Fig. 3A). In addition, these tissues were characterized by decreased relative ETR (Fig. 3B) and increased NPQ (Fig. 3C).

Increased infestation with eriophyid mite *Eriophyes padi* galls on leaves of *Prunus padus* resulted in gradually decreasing both F_V/F_M (Fig. 4A) and relative ETR (Fig. 4B), as well as increasing NPQ (Fig. 4C). In contrast, infestation of leaves of *Acer saccharinum* with eriophyid mite *Vasates quadripes* did not affect chlorophyll fluorescence

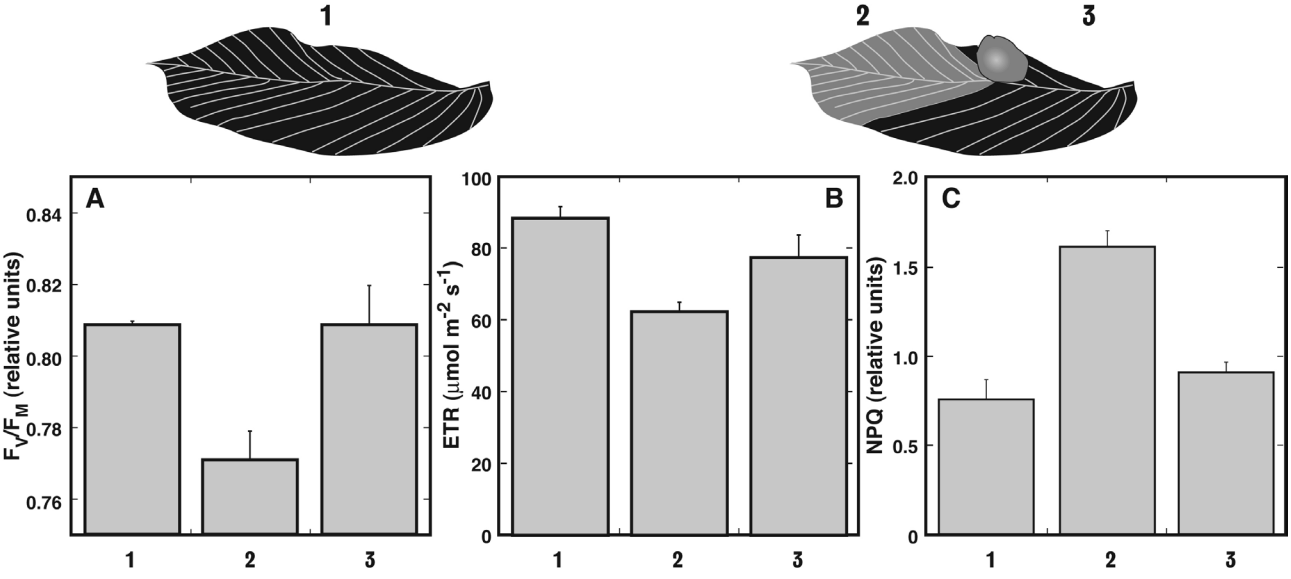

Fig. 3. Effect of a leaf vein pocket gall formed by *Colopha compressa* on chlorophyll *a* fluorescence characteristics in leaves of *Ulmus laevis*. Data are means ± SE from five leaves with five independent measurements per data point.

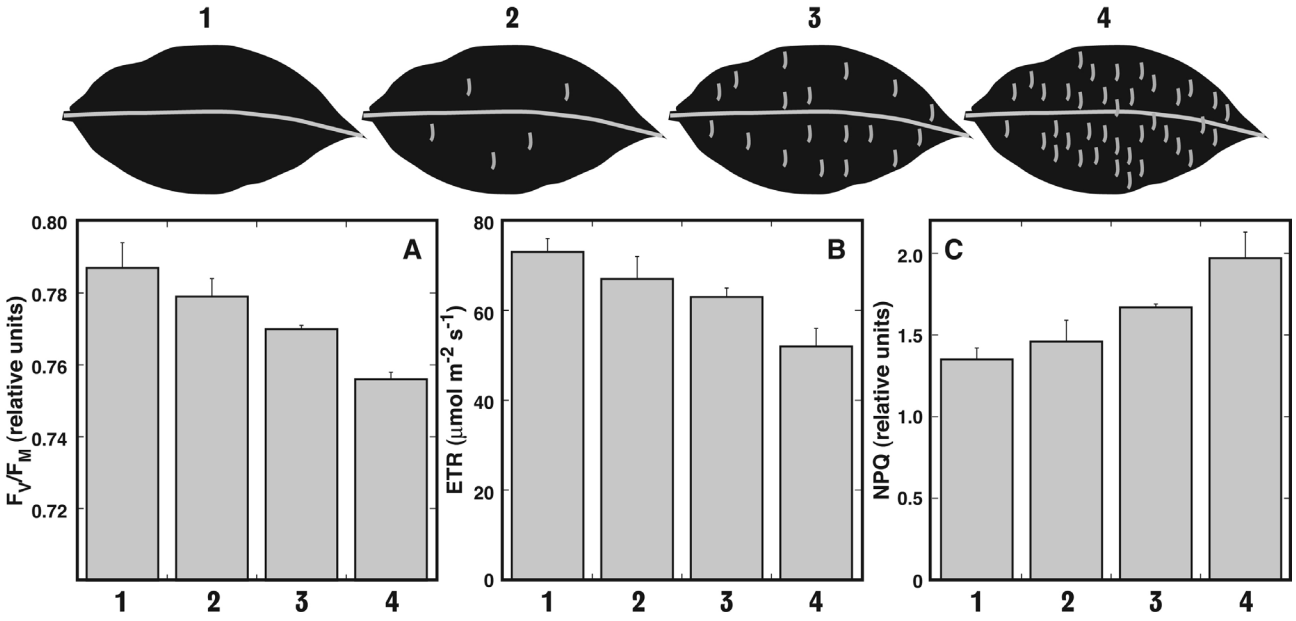

Fig. 4. Effect of degree of infestation with *Eriophyes padi* on chlorophyll *a* fluorescence characteristics in leaves of *Prunus padus*. 1, control without galls; 2, < 10 galls; 3, 10 to 20 galls; 4, > 20 galls. Data are means ± SE from five leaves with three independent measurements per data point.

characteristics (Fig. 5). Similarly, no significant effect on photochemistry of photosystem II was evident in leaves of *Tilia platyphyllos* infested with eriophyid mite *Eriophyes tiliae* (Fig. 6).

Maximum efficiency of photosynthesis (F_V/F_M) tended to decrease only in the tip tissues of control leaves of *Salix fragilis* (Fig. 7A). Relative ETR significantly decreased in these tissues (Fig. 7B) with no concomitant changes in NPQ (Fig. 7C). Infestation with tenthredinid sawfly *Pontania vesicator* resulted in significant decrease both in F_V/F_M (Fig. 7A) and ETR (Fig. 7B) in all parts of *S. fragilis* leaves with the strongest effect in the tip tissues. Similarly, higher NPQ was observed in tip tissues of infested leaves of *S. fragilis* (Fig. 7C). Both F_V/F_M (Fig. 8A) and ETR (Fig. 8B)

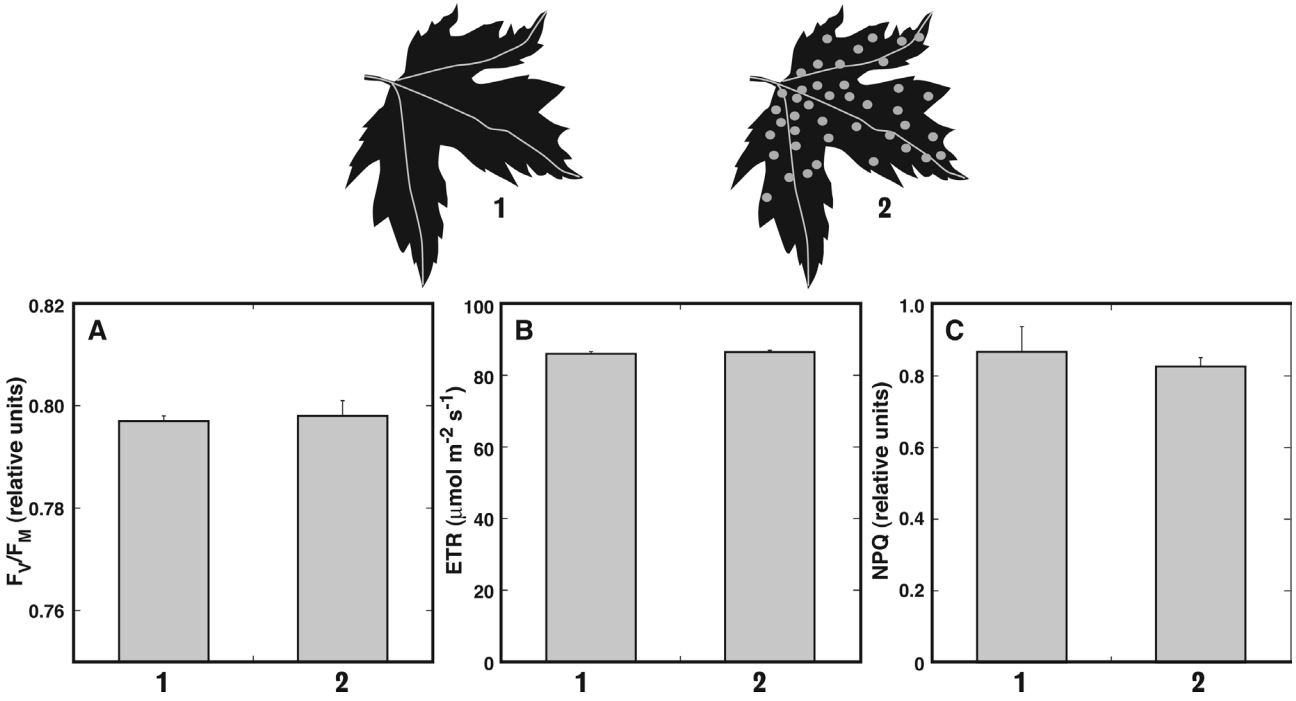

Fig. 5. Chlorophyll *a* fluorescence characteristics in leaves of *Acer saccharinum* infested with *Vasates quadripes*. Data are means ± SE from five leaves with three independent measurements per data point.

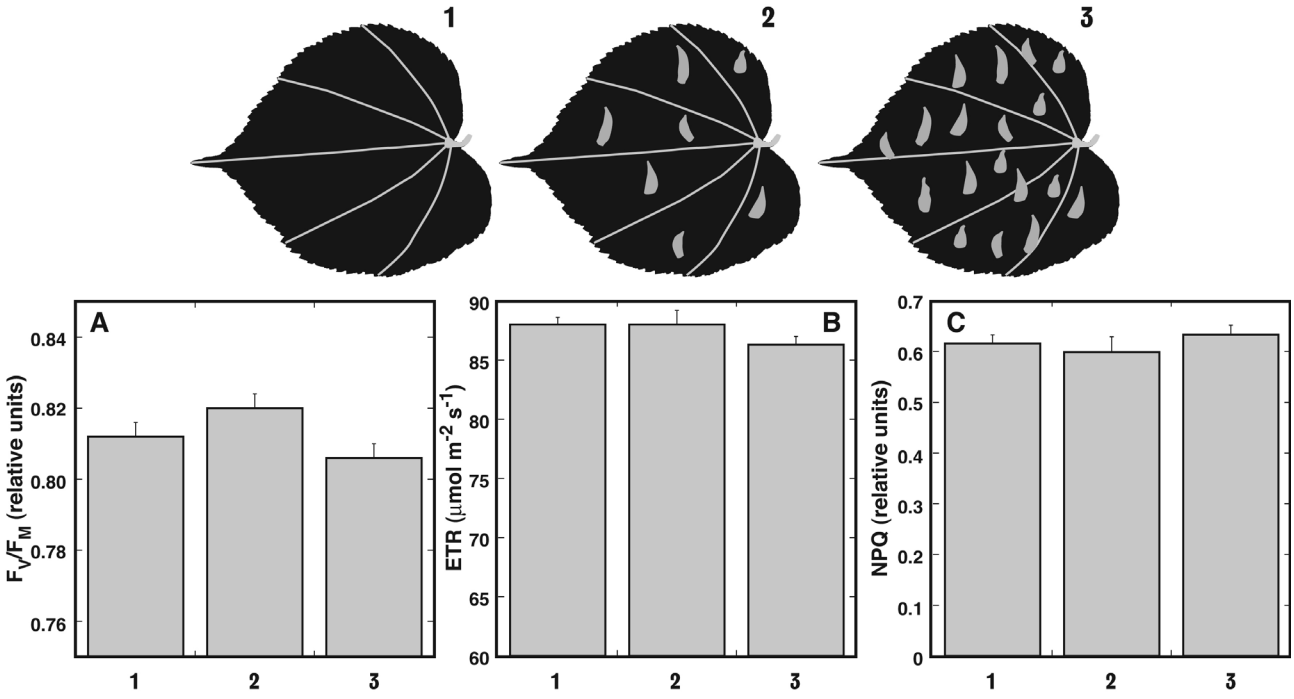

Fig. 6. Chlorophyll *a* fluorescence characteristics in leaves of *Tilia platyphyllos* infested with *Eriophyes tiliae*. Data are means ± SE from five leaves with three independent measurements per data point. 1, control without galls; 2, < 10 galls; 3, > 10 galls.

were lower in leaves having two galls, in comparison with leaves having only one gall. However, this relationship was not evident in more heavily infested leaves. NPQ increased with degree of infestation from one to two galls per leaf and decreased with further increase of gall number (Fig. 8C).

The presence of eriophyid mite *Eriophyes tetanotrix* galls on leaves of *Salix fragilis* alone or in combination with *Pontania*-induced galls had no effect on chlorophyll fluorescence parameters (data not shown).

Oxidative enzyme activity and ethylene production

Degree of infestation with tenthredinid sawfly *Pontania vesicator* on leaves of *Salix fragilis* was linearly correlated with peroxidase (R = 0.97) and polyphenol oxidase activity (R = 0.95) in leaf tissues (Fig. 9). Extractable protein content also increased in parallel with the degree of infestation (R = 0.83; data not shown). The presence of eriophyid mite galls on leaves of *S. fragilis* alone or together with galls by *P. vesicator* did not differ in oxidative enzyme activity (not shown). Similarly, oxidative enzyme activity in leaves of *Tilia platyphyllos* was not affected by the intensity of infestation with eriophyid mite galls (not shown). In contrast, peroxidase activity increased in leaves of *Acer saccharinum* in relation to number of eriophyid mite galls (Fig. 10).

The presence of aphid *Pemphigus spirothecae*-induced petiole spiral galls resulted in significant decrease of both peroxidase (Fig. 11A) and polyphenol oxidase (Fig. 11B) activity in leaf blades of *Populus nigra*. However, oxidative enzyme activity did not significantly differ between galled and un-galled petiole tissues of the galled leaf. Gall formation resulted in significant increase in ethylene production from leaf blade tissues, but the increase was only by 45% (Fig. 11C).

The presence of *Colopha compressa* leaf vein pocket galls on leaves of *Ulmus laevis* resulted in more than two-fold increase of ethylene production rate in affected leaf tissues over the control rate (Fig. 12). Ethylene production was not significantly affected in the basal part of the galled leaf. Gall tissues produced only a minor amount of ethylene.

Other five gall-inducer arthropod-host plant combinations were tested for a possible effect of galls on basal and detachment-induced rate of ethylene production from leaf tissues (Table 2). A 2.7-fold and 2.1-fold increase in ethylene production was found for intact *Ulmus glabra* leaves, infested with aphids *Eriosoma ulmi* and *Tetraneura ulmi*, respectively, compared to control leaves. In the other combinations tested, leaf galling did not result in changes of ethylene production from intact leaves. Also, a 6.6-fold and 6.2-fold increase was found in appropriate detached parts of *Ulmus glabra* leaves infested with aphid *Eriosoma ulmi* and in *Ulmus laevis* leaves infested with eriophyid mite *Aceria brevipunctata,* respectively, compared to control leaves. In the other gall-inducer–host plant combinations, the ethylene production rate was similar for control and galled leaf tissues. Gall tissues (except leaf-roll galls on *Ulmus glabra*) produced only negligible amounts of ethylene (0.004 to 0.008 nmol h^{-1} g^{-1}).

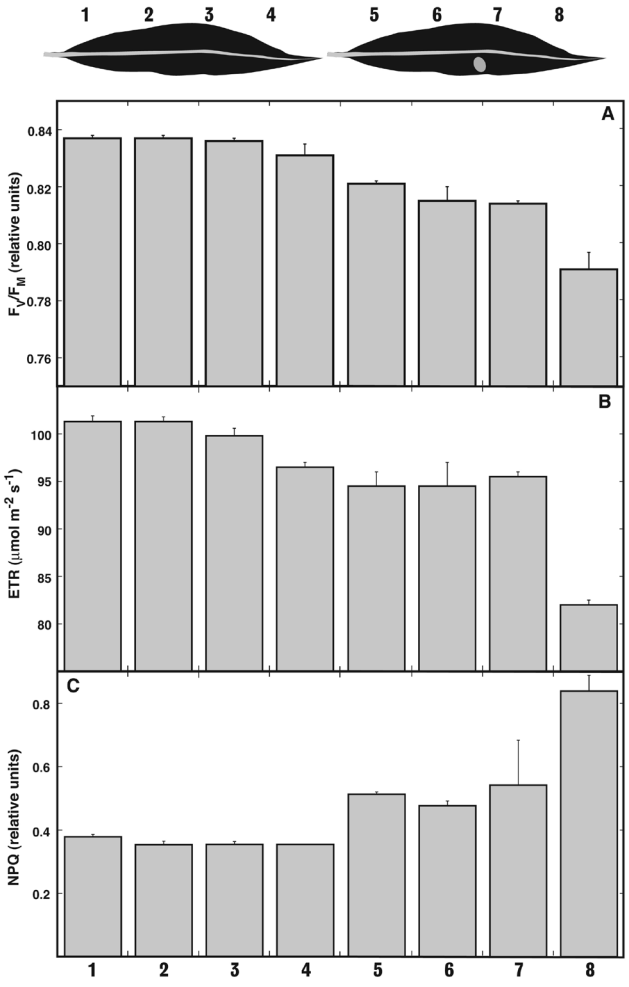

Fig. 7. Gradient of chlorophyll *a* fluorescence characteristics in leaves of *Salix fragilis* in relation to infestation with *Pontania vesicator*. Data are means ± SE from five leaves with two independent measurements per data point.

Discussion

Our previous study with nine host plant–gall-inducing arthropod combinations showed that lower photosynthetic pigment content in gall tissues, in comparison with the respective control leaf tissue, is a general phenomenon (Gailite et al. 2005a). In addition, neoplastic tissue contained significantly lower defense-related enzyme activity, with the exception of cecidomyidae-induced rosette galls on *Salix caprea*. Apart from biochemical changes in gall tissue, the adjacent non-galled tissue of infested leaves is of special interest due to the adaptive role for a gall-inducer. Relatively high oxidative enzyme activity in plant tissues can offer better protection against both pathogens and herbivores. In plant biotic interactions, both peroxidase and polyphenol oxidase act as antinutritional proteins, possibly interacting with gut phenolics, forming reactive electrophyle species, and in further negatively interfering with essential gut proteins (Zhu-Salzman et al. 2008; Barbehenn et al. 2010). In the context of the present study, it is important to determine if gall-induced changes in photosynthesis in adjacent tissues are related to defense enzyme activity, and if ethylene is an endogenous defense signal. More specifically, the possible effect of gall density on photosynthetic performance and defense response needs to be considered.

Complex physiological relationships between the gall-inducer and host plant tissues were observed for interactions of aphid-induced leaf vein pocket galls on *Ulmus laevis* and leaf-roll galls on *Ulmus glabra*. In both cases, relatively large parts of leaves were affected, either below the gall on leaves of *Ulmus glabra* or within the rolled-up part of leaves of *Ulmus laevis*. Within these tissues, dramatically decreased levels of photosynthetic pigments chlorophyll *a*, chlorophyll *b* and carotenoids (Gailite et al. 2005a) was

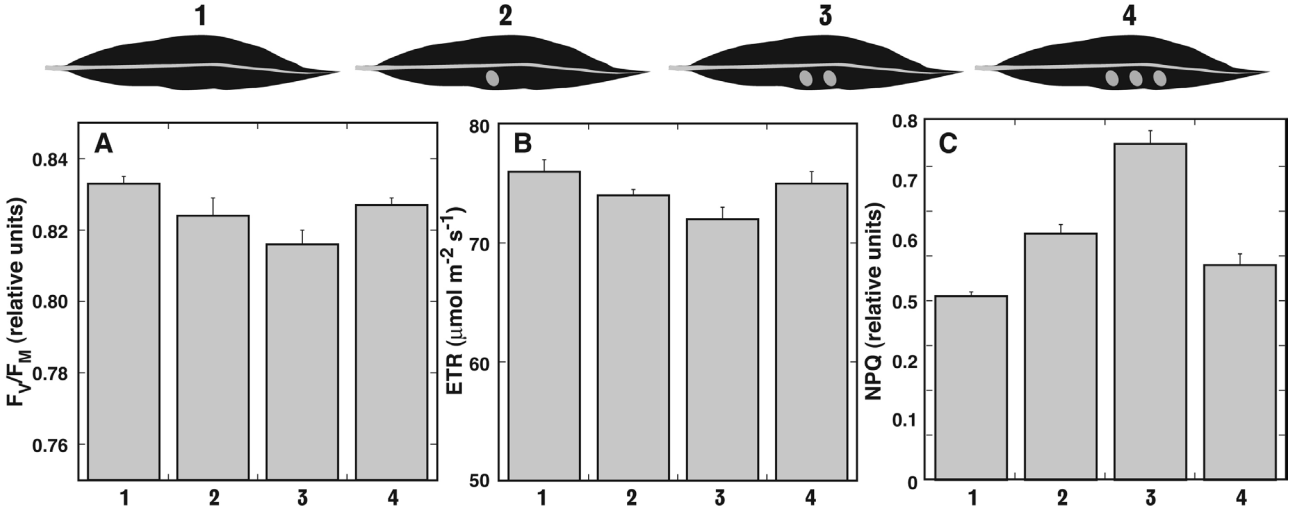

Fig. 8. Effect of degree of infestation with *Pontania vesicator* on chlorophyll *a* fluorescence characteristics in leaves of *Salix fragilis*. Data are means ± SE from five leaves with five independent measurements per data point.

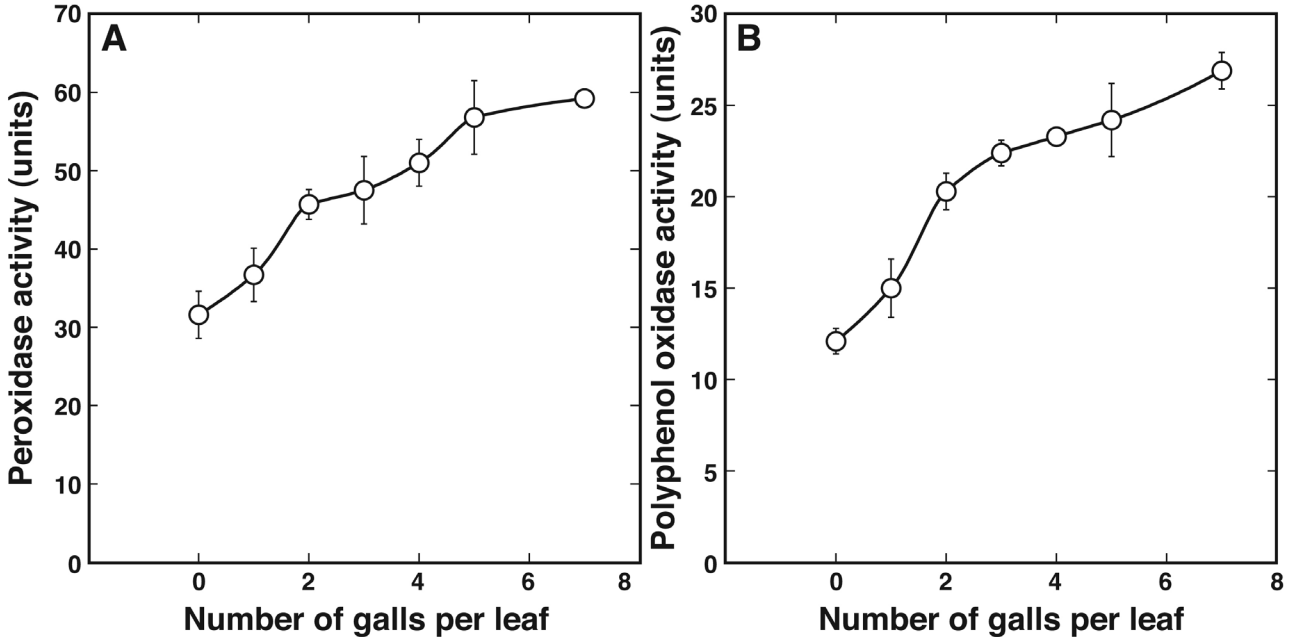

Fig. 9. Effect of degree of infestation with *Pontania vesicator* on peroxidase (A) and polyphenol oxidase (B) activity in leaves of *Salix fragilis*. Data are means ± SE from three to five individual leaves per data point..

associated with decreased photochemical performance (or even direct damage to photosystem II and photoinhibition of photosynthesis as in the case of *Ulmus laevis*; Fig. 2, 3), increased ethylene production in the affected tissues (Fig. 12; Table 2) and reduced activity of polyphenol oxidase with no apparent changes in peroxidase activity (Gailite et al. 2005a).

Photochemistry of photosynthesis was also significantly affected in leaves of *Prunus padus* heavily infested with eriophyid mite galls (Fig. 4), and in leaves of *Salix fragilis*, infested with tenthredinidae sawfly (Fig. 7, 8).

In some gall-inducer–host tree combinations, the degree of infestation affected leaf photosynthesis and defense-related characteristics. An increasing degree of infestation with eriophyid mite galls progressively decreased photosynthetic performance in leaves of *Prunus padus* (Fig. 4) but this effect was not evident for mite-induced galls on *Acer saccharinum* (Fig. 5) and *Tilia platyphyllos* (Fig. 6). To a certain extent, the gall density-dependent inhibitory effect on photochemistry of photosynthesis was characteristic also for *Salix fragilis* infested with *Pontania vesicator* (Fig. 8). Inhibition of photosynthesis was not causally related to defense enzyme activity, as defense enzymes in *Acer saccharinum* leaves were activated by infestation severity (Fig. 10) without any concomitant effect on photosynthesis (Fig. 5). However, in *Salix fragilis*, higher gall density-dependent oxidative enzyme activity was indeed associated with lower efficiency of photosynthetic performance. In addition, chlorophyll content was lower in leaves of *Salix fragilis* with two or three galls of *Pontania vesicator,* in comparison with single-galled or control leaves (Samsone et al. 2007). In contrast, in *Tilia platyphyllos*, no effect of the degree of infestation with eriophyid mites on chlorophyll content (Samsone et al. 2007) was evident.

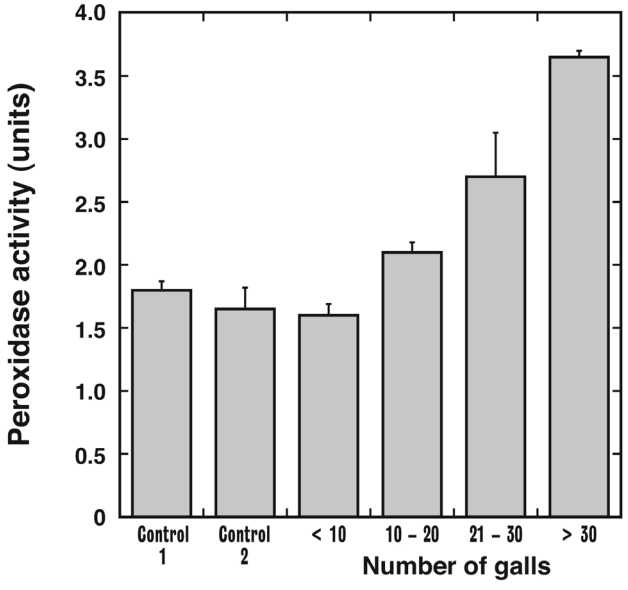

Fig. 10. Effect of degree of infestation with *Vasates quadripes* on peroxidase activity in leaves of *Acer saccharinum*. As a control, a leaf from uninfested branch (Control 1) and an uninfested leaf from infested branch (Control 2) were used. Data are means ± SE from four individual leaves per data point.

It was proposed previously that gall-inducer–dependent changes in chlorophyll a level and peroxidase activity in gall tissues are both controlled by common endogenous signals (Gailite et al. 2005a). Functioning of the gall involves an effect of mechanical damage to various degrees and/or arthropod-derived chemical signals, both affecting

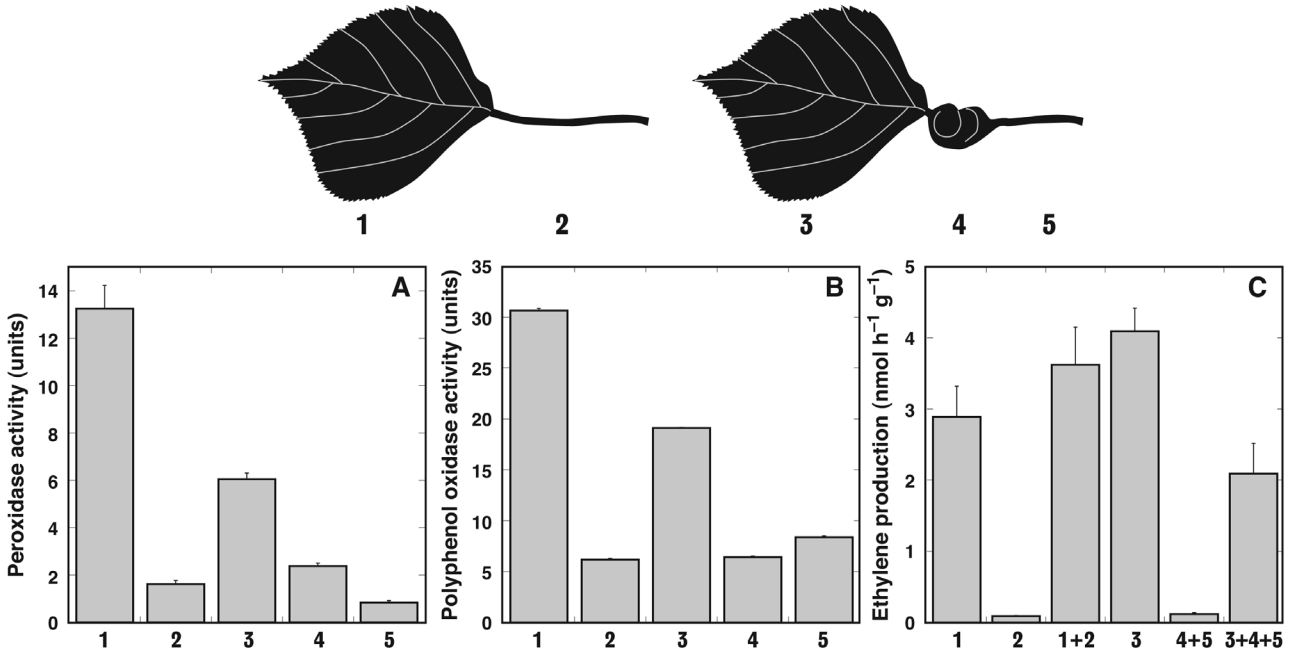

Fig. 11. Effect of infestation with *Pemphigus spirothecae* on peroxidase (A), polyphenol oxidase (B) activity and ethylene production (C) in leaves of *Populus nigra*. Data are means ± SE from independent measurements of five individual leaves.

endogenous plant signals and, consequently, physiology of host plants (Raman 2007). In non-galled plant tissues in close vicinity to galls, putative changes of metabolism are possibly due to the plant-derived sub-systemic signals. Only one study so far has shown that gall formation involves a systemic signal (Sopow et al. 2003) although no evidence of the nature of the signal was proposed. It has been shown also that insect herbivore-derived elicitors in plant tissues do not move systemically (Truitt, Pare 2004). Most evidently, differences between various gall-inducer–host plant combinations reflect various degree of physical and chemical interference of gall functioning on leaf physiology. Action of specific gall-inducer–associated signals was suggested by the *Pontania*-induced gall density effect on photosynthetic performance (Fig. 8) and defense enzyme activity (Fig. 9) in *Salix fragilis*. However, the presence of eriophyid mite galls alone or in combination with *Pontania* galls had no additional effect. On the other hand, the present study shows that specificity of endogenous sub-systemic signals are important for gall-related changes in non-galled leaf tissues, as increased ethylene production was concomitant to inhibition of photochemistry of photosynthesis in leaves of *Ulmus glabra* and *Ulmus laevis*.

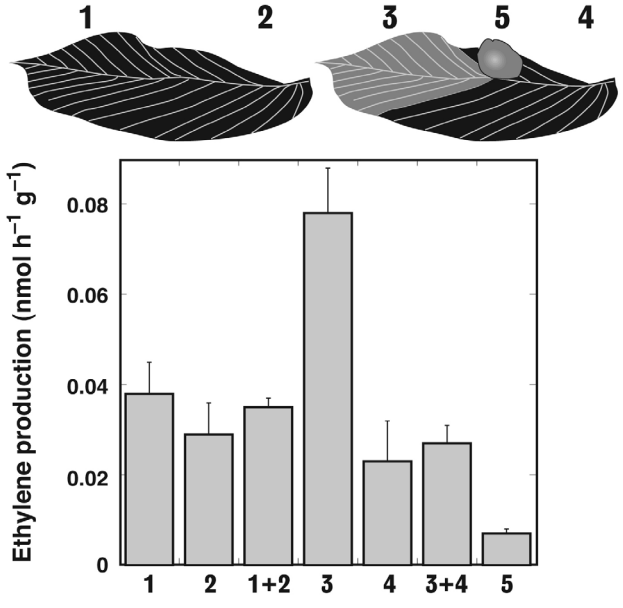

Fig. 12. Effect of infestation with *Colopha compressa* on ethylene production in leaves of *Ulmus laevis*. Data are means ± SE from independent measurements of five individual leaves.

The relationship between defense responses and photosynthesis near gall tissues may be explained by several mechanisms. Firstly, enhanced levels of defense-related jasmonate can directly lead to inhibition of photosynthesis (Reinbothe et al. 1993) after herbivore attack through reduced transcription of Rubisco, the primary enzyme of carbon fixation. This may result in increased non-photochemical quenching and, consequently, reduction of photosynthetic efficiency of photosystem II. However, increased NPQ together with decreased ETR but without strong decrease of F_V/F_M, was evident in leaves of *Salix fragilis* infested with *Pontania vesicator*, and the effect depended on the severity of infestation (Fig. 7, 8). Similarly, efficiency of photochemical reactions was depressed in *Eriosoma ulmi*-induced roll galls on leaves *Ulmus glabra* without significant decrease in F_V/F_M (Fig. 2).

Table 2. Ethylene production intensity (nmol h^{-1} g^{-1}) in different tissues of various host plant–gall-forming arthropod combinations. Data are means ± SE from five independent measurements. Different letters indicate significant differences for a particular gall-inducer–host plant combination ($p < 0.05$)

Combination	Control leaf (intact)	Galled leaf (intact)	Control leaf (detached part)	Galled leaf (detached non-galled part)	Galled leaf (detached galled part)
Prunus padus / *Eriophyes padi*	0.026 ± 0.006a	0.028 ± 0.009a	0.104 ± 0.020b	0.119 ± 0.024b	0.091 ± 0.018b
Salix fragilis / *Pontania vesicator*	0.078 ± 0.011a	0.078 ± 0.009a	0.076 ± 0.008a	0.059 ± 0.012a	0.055 ± 0.010a
Ulmus glabra / *Eriosoma ulmi*	0.082 ± 0.019a	0.224 ± 0.040b	0.109 ± 0.006a	0.072 ± 0.020a	0.721 ± 0.065c
Ulmus glabra / *Tetraneura ulmi*	0.093 ± 0.015a	0.195 ± 0.026b	0.114 ± 0.017a	0.091 ± 0.016a	0.109 ± 0.021a
Ulmus laevis / *Aceria brevipunctata*	0.051 ± 0.018a	0.065 ± 0.019a	0.072 ± 0.026a	0.066 ± 0.017a	0.448 ± 0.061b

Another mechanism might be up-regulation of oxidative metabolism during plant-herbivore interaction involving both ethylene and reactive oxygen species (ROS) signaling (Steinite et al. 2004). It is evident that light-dependent chloroplast-generated ROS species are involved in the positive control of defense responses against biotic agents (Roberts, Paul 2006). A recent study with a gall midge Hessian fly indicated that class III peroxidase-generated ROS is necessary for resistance against the insects (Liu et al. 2010). Similarly, hypersensitive response is a more likely mechanism of resistance against other leaf galling insects (Fernandes et al. 2003). Chloroplastic ROS as the components of stress-generated oxidative stress, in turn, can cause direct oxidative damage to different components of photosynthetic machinery (Murata et al. 2007). As a result, overall efficiency of photosynthesis is reduced, as suggested by decreased ETR concomitant with increased NPQ. This effect most likely can explain the drastic decrease of F_V/F_M in a gall-affected tissue of leaves of *Ulmus laevis* (Fig. 3) and of *Prunus padus* leaves heavily infested with *Eriophyes padi* (Fig. 4). Similar response of photochemistry was noted for tissues surrounding feeding sites of several gall-inducers, where areas of depressed photosynthetic efficiency were observed at various distances up to 14 mm (Aldea et al. 2006). When exploring the idea that gall-produced ROS leads to damage of photosynthetic machinery in gall tissues, it was shown that occurrence of numerous large plastoglobules in chloroplasts of galled tissues were related to oxidative stress and subsequent recovery of the thylakoid membrane system (de Oliveira et al. 2010). Maximum ETR was maintained at equally high levels in gall tissues vs. non-galled leaves for a particular gall-inducer–host plant combination. However, in other combinations, life-time deficiency of several pigment-protein complexes of the light-harvesting complex of photosystem II in gall tissues could account for decreased photosynthetic function at the level of light-harvesting, energy transfer and photochemical energy conversion (Yang et al. 2007; Huang et al. 2009). As a third mechanism, autotoxicity of several defense-related compounds has been reported to lead to reduction of photosynthetic efficiency (Gog et al. 2005). This mechanism could lead to changes in photochemical reactions similar to those discussed above for chloroplast-generated ROS. In the present study, it is difficult to evaluate the degree of participation of defense compounds in direct inhibition of photosynthetic machinery in galled tissues. It seems that this effect might be relevant in the gall-inducer–host plant combinations where the increased degree of infestation caused both increase of defense enzyme activity and decrease of F_V/F_M, as in the case of *Pontania vesicator* galls on *Salix fragilis*.

It can not be ruled out that decrease in CO_2 uptake due to gall functioning affects photosynthetic performance. However, no experimental evidence so far has confirmed negative effect of CO_2 uptake in response to gall formation. On the contrary, leaves of *Prunus serotina* galled by eriophyid mite *Phytoptus cerasicrumena* were observed to have increased conductance and internal CO_2 concentration in comparison to control leaves, in spite of significantly reduced photosynthesis (Larson 1998). Also, decreased photochemical efficiency around galls was not found to be associated with stomatal closure, as suggested by lower surface temperature in affected tissues indicating increased transpiration (Aldea et al. 2006).

It is evident, that these mechanisms operate to different degrees in different gall-inducer–host plant combinations. Depression of photosystem II operating efficiency was found to occur only in close vicinity to a galled site in the majority of cases, propagating up a distance of only a few millimeters (Aldea et al. 2006). The depression was only partially related to photoinhibition of photosynthesis, but was associated with increased NPQ. Most importantly, photochemistry of photosynthesis was not affected in the remaining tissues of the galled leaf. In the present study, even relatively distant tissues of galled leaves could be affected by gall localization at the level of ethylene production and photochemistry of photosynthesis.

A recent study with 22 different forms of biotic damage on eight different plant species showed that depression of photosynthesis-related gene expression after biotic damage is indeed an universal defense response in plants, together with upregulation of genes coding for jasmonate synthesis and both salicylic acid and ethylene-response genes (Bilgin et al. 2010). However, no data on galling arthropods were

included in the study. It is highly likely that apart from compensatory increase in photosynthetic rate in distant tissues in the cases of chewing herbivores and cynipid gall-inducers (Fay et al. 1993), there is a general relationship between high (induced) state of defense responses and inhibition of photosynthesis in local tissues during plant-herbivore interaction. Even without herbivore activity, high concentration of defense chemicals of phenolic nature is associated with lower photosynthetic rate and leaf longevity and vice versa, as in trees of Betulaceae family (Koike et al. 2006).

There is no information available in the literature on a possible involvement of ethylene as an endogenous regulator in control of arthropod gall-inducer–host plant interactions. As gall-inducer feeding involves wounding and there are appropriate changes in defense responses, ethylene is one of the possible candidates in control of plant responses to galls. Ethylene is known to participate in plant responses to herbivore feeding through the control of appropriate defense-related gene expression at all levels of plant-herbivore interactions (von Dahl, Baldwin 1997; Steinite et al. 2004). Herbivore oviposition effects are clearly herbivore-host plant specific, as both induction and suppression of plant defense responses can occur. Defense responses are induced in pine trees by oviposition of sawfly herbivore concomitant with reduced photosynthetic activity (Schroder et al. 2005) and inhibition of ethylene emission (Schroder et al. 2007). Oviposition is shown to suppress direct plant defense responses against chewing herbivores (Bruessow et al. 2010). Specificity of response to generalist vs. specialist herbivore feeding is also controlled by ethylene. On the other hand, ethylene is a necessary endogenous signal in *Agrobacterium tumefaciens*-induced plant tumour establishment (Wächter et al. 1999).

In the present study, effect of gall formation on ethylene production did not depend on type of the gall-inducer, as infestation with eriophyid mites did not affect ethylene production in *Prunus padus*, but stimulated it in *Ulmus laevis* (Table 2). Similarly, aphid gall-inducers increased ethylene production in affected leaves of *Ulmus glabra* (Table 2), while no significant effect was evident in *Populus nigra* (Fig. 11). It is tempting to hypothesize that increased ethylene production in the case of *Colopha compressa* galls on *Ulmus laevis* and *Eriosoma ulmi* galls on *Ulmus glabra* is causally related to downregulation of photosynthesis. Most likely, some indirect mechanism possibly involving an ethylene-ROS relationship (Steinite et al. 2004) might participate here, as it is argued that ethylene production and photosynthesis are positively correlated through the role of CO_2 in ethylene synthesis (Khan 2006). However, contrary to the general idea that high ethylene production during plant-herbivore interaction is responsible for induction of defense enzymes (Ohme-Takagi et al. 2000; Steinite et al. 2004; Gailite et al. 2005b), polyphenol oxidase activity was obsrved to be reduced in 17 – 20% of galled tissues of respective plants with no concomitant changes in peroxidase activity (Gailite et al. 2005a).

It would be interesting to test experimentally if high ethylene production is related to photoinhibition of photosynthesis and defense responses, as in the gall-affected part of the leaf of *Ulmus laevis*. The use of inhibitors of ethylene formation and ethylene perception would allow to manipulate both ethylene production and ethylene signaling and to monitor if this affects photochemistry of photosynthesis and oxidative enzyme activity.

In conclusion, the presence of galls on leaves differentially affects host plant photosynthesis and putative defense characteristics. Variable effect of gall formation on host plant photochemistry of photosynthesis and oxidative enzyme activity revealed in the present study might be explained by specific gall-inducer–related signals at the site of activity in combination with specific endogenous plant signals involving ethylene. In some gall-inducer–host plant combinations, increasing intensity of infestation leads to enhanced responses, possibly through production of a stronger level of putative gall-inducer–related and/or plant endogenous signals.

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