

Chapter 11

Ozone affects leaf surface–pest interactions

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Abstract

Tropospheric ozone (O₃) levels are increasing around the world and damaging concentrations now occur in 25% of the world forests. This study was conducted at an open air CO₂ and O₃ exposure (Aspen FACE) facility in northern Wisconsin. Here, we present evidence for a link between long-term, low-level O₃ exposure and alterations in trembling aspen (*Populus tremuloides* Michx.) epicuticular waxes resulting in consequential changes to leaf surface properties. In turn, these changes have resulted in increased incidence of natural infection by the aspen leaf rust (*Melampsora medusae* Thuem. f. sp. *tremuloidae*). These results have been consistent over 3 years varying in natural rust occurrence on three trembling aspen clones differing in O₃ sensitivity. The presence of elevated CO₂ did not alleviate the O₃ effects.

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1. Introduction

The global atmospheric CO₂ concentration has risen by nearly 30% since the preindustrial time period (IPCC, 2001). This increase is likely largely due to increasing industrial emissions and global deforestation (Keeling et al., 1995). Similarly, emissions of oxidized nitrogen (NO_x) and volatile organic compounds from fossil fuel emissions related to human activities, such as industrial production and transportation, have resulted in large increases in background tropospheric O₃ concentrations (Finlayson-Pitts and Pitts, 1997; Stevenson et al., 1998; IPCC, 2001). Fowler et al. (1999a, 1999b) suggest that nearly one-quarter of the earth's forests are currently at risk from tropospheric O₃ where the July peak concentrations exceed 60 ppb.

Elevated CO₂ and O₃ impact aspen (*Populus tremuloides* Michx.) trees in diametrically opposite ways. Elevated CO₂ stimulates photosynthesis (Tjoelker et al., 1998; Noormets et al., 2001a, 2001b), delays foliar senescence in autumn (Karnosky et al., 2003), and stimulates aboveground (Norby et al., 1999) and belowground (King et al., 2001; Kubiske and Godbold, 2001) growth. Trees grown under elevated CO₂ generally have lower nitrogen concentrations in their foliage, lower Rubisco (ribulose biphosphate carboxylase) concentrations (Moore et al., 1999), altered defense compounds (Lindroth et al., 1993, 1997) and decreased levels of antioxidants (Wustman et al., 2001).

In contrast to the largely beneficial effects of CO₂, O₃ is generally detrimental to aspen growth and productivity. Ozone has been shown to induce foliar injury (Karnosky, 1976), decrease foliar chlorophyll content (Gagnon et al., 1992), accelerate leaf senescence (Karnosky et al., 1996), decrease photosynthesis (Coleman et al., 1995a), alter carbon allocation (Coleman et al., 1995b), alter epicuticular wax production (Percy et al., 2002) and chemical composition (Karnosky et al., 2002; Percy et al., 2002), and decrease above- and belowground growth (Wang et al., 1986a, 1986b; Karnosky et al., 1996; Isebrands et al., 2001). Extrapolation of open-top chamber data from O₃ impacts on aspen to the natural aspen range suggests that 14–33% biomass loss may be occurring over 50% of their distribution in the eastern US (Hogsett et al., 1997). Of significance to industry, is the fact that the aspen resource is now almost fully utilized in many parts of North America (Tuskan and Walsh, 2001).

Current climate change scenarios predict further increases in CO₂ (Stott et al., 2000; IPCC, 2001) and O₃ (Stevenson et al., 1998; Fowler et al., 1999a, 1999b) over the next century. Because little research has been done on the impacts of these interacting pollutants and conflicting results have been found even for the same species, it is difficult to predict how future forests will respond to these interacting pollutants. What is known, particularly from our work on Aspen FACE, is that the negative roles of insects and disease are

likely to become more important under co-exposure to both greenhouse gases (Percy et al., 2002). In this paper, we present evidence of the impact of O₃ on aspen leaf surfaces and we link these changes to increased occurrence of aspen leaf rust caused by *Melampsora medusae*.

2. Methods

2.1. The aspen FACE experiment

This experiment was conducted at the FACTS-II (Aspen FACE) site located on USDA Forest Service land near Rhineland, WI (Karnosky et al., 1999; Dickson et al., 2000). The experiment includes a full factorial with 12 30 m diameter treatment rings with three control rings, three rings with elevated O₃, three rings with elevated CO₂, and three rings with elevated O₃ + elevated CO₂. All rings are a minimum of 100 m apart. The rings were planted in late summer 1997 and treatments ran from aspen bud-break to bud-set in 1998, 1999, 2000, 2001, and 2002. The eastern half of each ring was randomly planted at 1 m × 1 m spacing in two tree plots with five aspen clones differing in O₃ tolerance (8L, 216 and 271 = relatively tolerant and 42E and 259 = relatively sensitive). The northwestern quadrant of each ring was planted at the same spacing with alternating aspen clone 216 and sugar maple seedlings, and the southwestern quadrant of each ring was planted as above with aspen clone 216 and paper birch seedlings.

CO₂ and O₃ were administered during the daylight hours with elevated CO₂ being targeted for 560 ppm, which is 200 ppm above the daylight ambient CO₂ concentration. O₃ is administered at a target of 1.5 × ambient and is not administered during periods of cold weather, or when plants are wet from fog, dew, or rain events. Additional details on the experimental design and pollutant generation and monitoring can be found in Karnosky et al. (1999), Dickson et al. (2000), and Karnosky et al. (2003).

2.2. The aspen clones

Essential information on the three aspen clones investigated is listed below in Table 1.

2.3. Epicuticular wax physicochemical characteristics

To characterize epicuticular wax (EW) production, five recently mature (LPI 8–12) leaves from major lateral branches from each of five trees per clone, per FACE ring, were collected and pooled for analysis. Wax was removed with

Table 1. *Populus tremuloides* clones used in this study

Clone	Origin state (county)	O ₃ tolerance ^a	Rust susceptibility ^b (low O ₃)	Rust susceptibility ^b (high O ₃)	O ₃ -induced stomatal occlusion ^c
216	Wisconsin (Bayfield)	tolerant	low	low	moderate
259	Indiana (Porter)	susceptible	moderate	high	high
271	Indiana (Porter)	tolerant	low	moderate	moderate

^aOzone tolerance by visible foliar injury (Berrang et al., 1991), photosynthesis responses (Coleman et al., 1995a, 1995b; Noormets et al., 2001a, 2001b) and growth responses (Isebrands et al., 2001).

^bFrom Karnosky et al., 2002.

^cFrom Karnosky et al., 1999, and Percy et al., 2002.

chloroform (Karnosky et al., 2002) and amount of wax deposit per unit leaf area calculated. Quantitative wax chemical composition was then determined using high-temperature capillary gas chromatography (Percy et al., 2002).

For examination of EW structure, leaf segments were excised, mounted on a stub, gold-coated on a cold stage and examined under a JSM 6400 SEM. Leaf surface EW and fungal (hyphae, spores, germ tubes) structures and density were quantified on abaxial leaf surfaces. Stomatal occlusion was quantified as described by Mankovska et al. (2003).

2.4. Leaf surface wettability

Aspen leaf rust occurs on abaxial leaf surfaces. Aspen clone leaf surface wettability was assessed by measuring abaxial leaf surface droplet contact angle. Segments from leaves used for SEM examination were excised and mounted onto glass slides using double-faced tape. Small volume (0.2 µl; ~ 700 µm diam.) deionized water droplets were detached from a glass syringe and equilibrium contact angles measured (1 degree) using an NRL Contact Angle Goniometer (Rame-Hart, Mountain Lakes, New Jersey).

2.5. Rust occurrence

Melampsora medusae Thuem. f. sp. *tremuloidae* was examined by observations of urediniospores with scanning electron microscopy and/or light microscopy. The percentage of infected leaves per tree for every tree in the core of each Aspen FACE ring ($n = 1839$ trees total) was estimated in mid to late August and the severity of rust occurrence was scored on an average of 20 leaves per tree from 1 to 5 with 1 = 1–20% of the leaf area covered with urediniospores, 2 = 21–40%, 3 = 41–60%, 4 = 61–80%, and 5 = 81–100%. An

index of mean severity of infection will then be calculated as percent leaves injured \times severity of rust occurrence (Karnosky et al., 2002; Percy et al., 2002).

3. Results and discussion

3.1. Epicuticular wax production and chemical composition

The leaf cuticle, and in particular the outermost epicuticular wax layer, forms an important defensive barrier against pests (Jeffree, 1996). The role of the cuticle as a physical barrier to protect leaves from fungal infection is well established (Mengden, 1996). Exposure of aspen clones to O_3 greatly altered epicuticular wax physicochemical characteristics. Under O_3 , leaves developed a significantly larger wax deposit than in the control treatment. Amount of EW recovered from leaves exposed to O_3 averaged $45.5 \mu\text{g cm}^{-2}$, or 23% greater than in the control ($36.8 \mu\text{g cm}^{-2}$) treatment (Fig. 1).

The structure of epicuticular wax is normally conferred by its chemical composition. In other words, aliphatic compounds that crystallize onto the leaf surface do so in a form determined by their inherent chemistry. For instance,

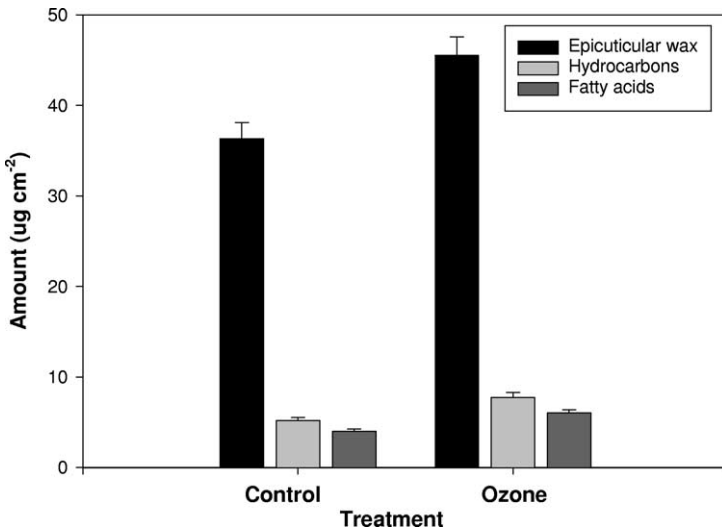


Figure 1. Effect of O_3 on *Populus tremuloides* epicuticular wax physicochemical characteristics. Amount of epicuticular wax recovered from aspen leaf surfaces and relative proportions of two major wax classes which influence surface wettability. Modified from Karnosky et al. (2002) and Percy et al. (2002).

primary alcohols crystallize into small irregular plates, secondary alcohols into hollow tubes (Jeffree et al., 1975). In a number of tree species, O_3 has been reported to act upon the two major biosynthetic pathways producing epicuticular wax and causing shifts in C allocation between them, thus altering chemical composition of the leaf surface, and by default, wax structure (Percy et al., 1994). In aspen, synthesis of the two largest wax classes (hydrocarbons, fatty acids) was stimulated under O_3 fumigation. Amount of hydrocarbons (carbon chains 24–33) was increased 48% and fatty acids (carbon chains 16–30) 51% relative to the controls (Fig. 1). Although hydrocarbons form crystalline plates conferring microroughness and greater hydrophobicity, fatty acids form amorphous films that increase leaf water retention. Also of interest was the reported (Karnosky et al., 2002) carbon chain lengthening in hydrocarbon leaf wax deposits under O_3 , however, this is likely of little importance in terms of leaf surface properties and fungal performance.

3.2. *Epicuticular wax structure*

To qualify the degree of epicuticular wax alteration following seasonal O_3 exposure we examined aspen leaf surfaces using SEM. When examining the O_3 sensitive clone 259, clear and consistent differences between the control and O_3 treated abaxial leaf surfaces were seen. Particularly in areas around stomata, a change from fine crystalline to amorphous (and more hydrophillic) structure was observed (Fig. 2).

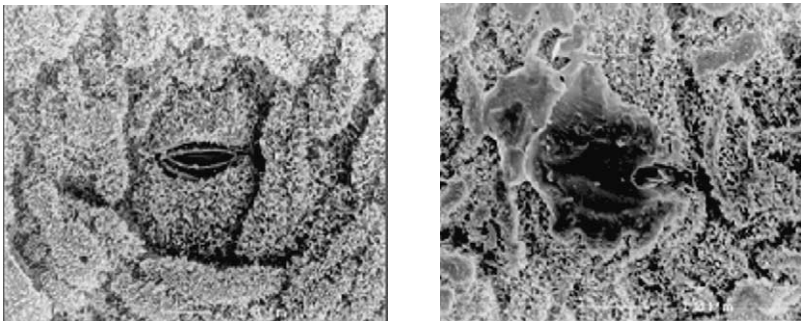


Figure 2. Effect of O_3 on epicuticular wax structure as seen under the SEM. On the left is a SEM of the lower leaf surface waxes and stomate of clone 259 under low O_3 conditions. On the right is a SEM of the same clone grown under $1.5\times$ ambient O_3 . Note the difference in wax structure and high level of stomatal occlusion (from Karnosky et al., 2003).

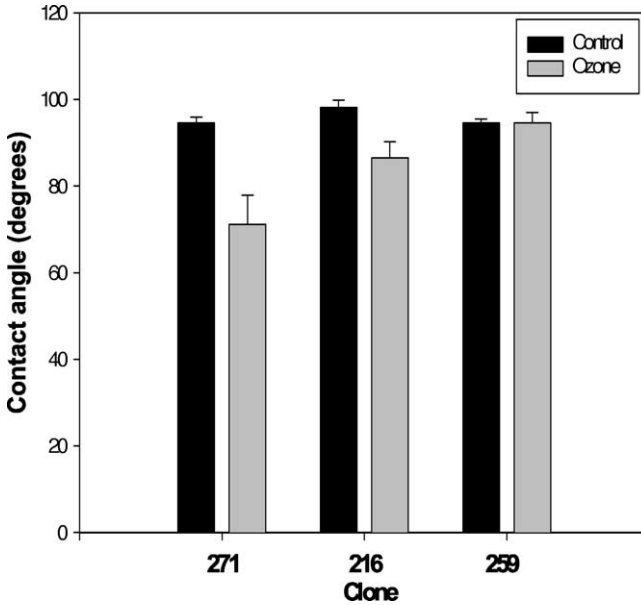


Figure 3. Leaf droplet contact angle (DCA) on abaxial leaf surfaces of three *Populus tremuloides* clones. Note the large decrease in DCA in the two O_3 tolerant clones. Modified from Karnosky et al. (2002).

3.3. Leaf surface wettability

Wettability of the leaf cuticle has a well-known role in influencing fungal spore survival (Kerstiens, 1996). As aspen rust is usually found on abaxial leaf surfaces, abaxial surface wettability has a particular significance in *Melampsora* uredinospores. As we reported earlier (Karnosky et al., 2002), aspen exposure to O_3 caused the abaxial leaf surfaces to become more wettable (decreased contact angle) in two of the three clones studied. Contact angles in the O_3 treatment were 25% lower in clone 271 and 12% lower in clone 216 relative to the controls Fig. 3). Both clones are considered to be O_3 tolerant (see Table 1) based on expression of visual symptoms. Interestingly, there was no difference in contact angle due to treatment in clone 259, which is known to be very sensitive to O_3 .

3.4. Rust occurrence

Allen (1991) has reported on the important role of topographic signals from the *Populus deltoides* (Bartr.) leaf surface on development of appressoria from

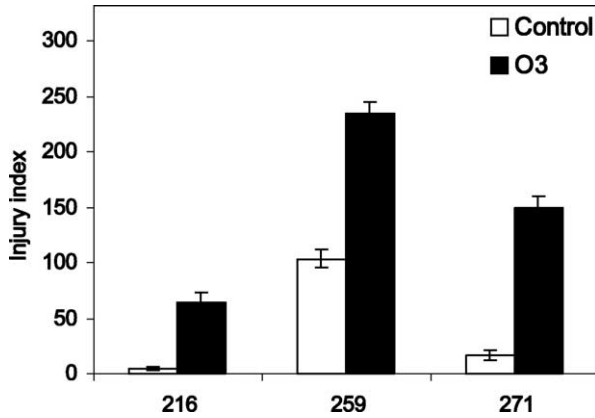


Figure 4. Effect of O₃ on aspen leaf rust infection index calculated for three aspen clones. Significantly higher (3–5×) levels of rust occurrence were seen in two clones due to O₃. Modified from Karnosky et al. (1999) and Karnosky et al. (2002).

urediniospores of *Melampsora medusae*. The optimum morphology would be expected to be similar to that we observed for the amorphous wax deposits (see Fig. 2(b)) developed under O₃ in this study. In our experiment, significant differences in rust infection index occurred due to treatment and clone (Fig. 4). Seasonal exposure to O₃ dramatically increased rust infection three- to five-fold in all clones. Increases were greatest for clone 271 and least in clone 216.

Our data support those of Beare et al. (1999) who also reported that O₃ increases *Melampsora* rust infection in *Populus*. In our clones, the increase in proportion of amorphous wax deposits (Fig. 2) and the observed alteration towards less crystalline wax deposits noted previously (Mankovska et al., 1998; Karnosky et al., 1999) on these aspen clones could have reduced ridge heights towards the optimum preferred for appressorium formation. Also, fatty acid deposits tend to be situated in areas of the leaf with reduced topography, so the concurrent increase in amount of more hydrophobic (Holloway, 1969) hydrocarbons we noted (Fig. 1) may not have been able to counteract the increased wettability due in large part to the increase in fatty acids following O₃ exposure.

4. Conclusions

Of special relevance to this investigation is the fact that modifications to epicuticular wax physicochemical characteristics and leaf surface properties we have documented above were induced by O₃ at a scale relevant to organisms

such as *Melampsora*. Our data show that O₃ can modify aspen leaf surfaces. Leaf morphology, microroughness and physicochemical characteristics of the outermost epicuticular wax layer in direct contact with the atmosphere influence key leaf surface properties such as wettability, water retention and foliar uptake (Percy and Baker, 1987, 1988). The microenvironment at the phylloplane is critical to the process of fungal infection (Mengden, 1996). In our opinion, one critical impact of low-level O₃ exposure over the long term may be an increased predisposition of aspen to diseases such as aspen leaf rust.

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References

- Allen, E.A., 1991. Appressorium formation in response to topographical signals by 27 rust species. *Phytopathology* 81, 323–331.
- Beare, J.A., Archer, S.A., Bell, J.N.B., 1999. Effects of *Melampsora* leaf rust disease and chronic ozone exposure on poplar. *Environ. Pollut.* 105, 419–426.
- Berrang, P.C., Karnosky, D.F., Bennett, J.P., 1991. Natural selection for ozone tolerance in *Populus tremuloides*: An evaluation of nationwide trends. *Can. J. Forest Res.* 21, 1091–1097.
- Coleman, M.D., Dickson, R.E., Isebrands, J.G., Karnosky, D.F., 1995a. Photosynthetic productivity of aspen clones varying in sensitivity to tropospheric ozone. *Tree Physiol.* 15, 585–592.
- Coleman, M.D., Dickson, R.E., Isebrands, J.G., Karnosky, D.F., 1995b. Carbon allocation and partitioning in aspen clones varying in sensitivity to tropospheric ozone. *Tree Physiol.* 15, 593–604.
- Dickson, R.E., Lewin, K.F., Isebrands, J.G., Coleman, M.D., Heilman, W.E., Riemenschneider, D.E., Sober, J., Host, G.E., Hendrey, G.R., Pregitzer, K.S., Karnosky, D.F., 2000. Forest atmosphere carbon transfer storage-II (FACTS II)—The aspen free-air CO₂ and O₃ enrichment (FACE) project in an overview. USDA Forest Service North Central Experiment Station. General Tech. Rep. NC-214.
- Finlayson-Pitts, B.J., Pitts Jr., J.N., 1997. Tropospheric air pollution: ozone, airborne toxics, polycyclic aromatic hydrocarbons, and particulates. *Science* 276, 1045–1051.
- Fowler, D., Cape, J.N., Coyle, M., Flechard, C., Kuylenstierna, J., Hicks, K., Derwent, D., Johnson, C., Stevenson, D., 1999a. The global exposure of forests to air pollutants. *J. Water Air Soil Pollut.* 116, 5–32.

- Fowler, D., Cape, J.N., Coyle, M., Smith, R.I., Hjellbrekke, A.G., Simpson, D., Derwent, R.G., Johnson, C.E., 1999b. Modelling photochemical oxidant formation, transport, deposition, and exposure of terrestrial ecosystems. *Environ. Pollut.* 100, 43–55.
- Gagnon, Z.E., Karnosky, D.F., Dickson, R.E., Isebrands, J.G., 1992. Effects of ozone on chlorophyll content in *Populus tremuloides*. *Amer. J. Bot.* 79 (Suppl.), 107.
- Hogsett, W.E., Weber, J.E., Tingey, D., Herstrom, A., Lee, E.H., Laurence, J.A., 1997. An approach for characterizing tropospheric ozone risk to forests. *Environ. Manag.* 21, 105–120.
- Holloway, P.J., 1969. Chemistry of leaf waxes in relation to wetting. *J. Sci. Food Agric.* 20, 124–128.
- IPCC (Intergovernmental Panel on Climate Change), 2001. A report of working group I of the Intergovernmental Panel on Climate Change. Available at <http://www.ipcc.ch/>.
- Isebrands, J.G., McDonald, E.P., Kruger, E., Hendrey, G., Pregitzer, K., Percy, K., Sober, J., Karnosky, D.F., 2001. Growth responses of *Populus tremuloides* clones to interacting carbon dioxide and tropospheric ozone. *Environ. Pollut.* 115, 359–371.
- Jeffree, C.E., 1996. Structure and ontogeny of plant cuticles. In: Kerstiens, G. (Ed.), *Plant Cuticles*. Bios Scientific Publishers, pp. 175–188 and pp. 33–82.
- Jeffree, C.E., Baker, E.A., Holloway, P.J., 1975. Ultrastructure and recrystallization of plant epicuticular waxes. *New Phytol.* 75, 539–549.
- Karnosky, D.F., 1976. Threshold levels for foliar injury to *Populus tremuloides* Michx. by sulfur dioxide and ozone. *Can. J. Forest Res.* 6, 166–169.
- Karnosky, D.F., Gagnon, Z.E., Dickson, R.E., Coleman, M.D., Lee, E.H., Isebrands, J.G., 1996. Changes in growth, leaf abscission, and biomass associated with seasonable tropospheric ozone exposures of *Populus tremuloides* clones and seedlings. *Can. J. Forest Res.* 16, 23–27.
- Karnosky, D.F., Mankovska, B., Percy, K., Dickson, R.E., Podila, G.K., Sober, J., Noormets, A., Hendrey, G., Coleman, M.D., Kubiske, M., Pregitzer, K.S., Isebrands, J.G., 1999. Effects of tropospheric O₃ on trembling aspen and interaction with CO₂: results from an O₃-gradient and a FACE experiment. *J. Water Air Soil Pollut.* 116, 311–322.
- Karnosky, D.F., Percy, K.E., Xiang, B., Callan, B., Noormets, A., Mankovska, B., Hopkin, A., Sober, J., Jones, W., Dickson, R.E., Isebrands, J.G., 2002. Interacting CO₂–tropospheric O₃ and predisposition of aspen (*Populus tremuloides* Michx.) to infection by *Melampsora medusae* rust. *Global Change Biol.* 8, 329–338.
- Karnosky, D.F., Zak, D.R., Pregitzer, K.S., Awmack, C.S., Bockheim, J.G., Dickson, R.E., Hendrey, G.R., Host, G.E., King, J.S., Kopper, B.J., Kruger, E.L., Kubiske, M.E., Lindroth, R.L., Mattson, W.J., McDonald, E.P., Noormets, A., Oksanen, E., Parsons, W.F.J., Percy, K.E., Podila, G.K., Riemenschneider, D.E., Sharma, P., Thakur, R., Sober, A., Sober, J., Jones, W.S., Anttonen, S., Vapaavuori, E., Mankovska, B., Heilman, W.E., Isebrands, J.G., 2003. Low levels of tropospheric O₃ moderate responses of temperate hardwood forests to elevated CO₂: a synthesis of results from the Aspen FACE project. *Funct. Ecol.* 17, 289–304.
- Keeling, C.M., Whort, T.P., Wahlen, M., Vander Plicht, J., 1995. International extremes in the rate of rise of atmospheric carbon dioxide since 1980. *Nature* 375, 666–670.
- Kerstiens, G., 1996. Barrier properties of the cuticle to water, solutes and pest and pathogen penetration in leaves of plants grown in polluted atmospheres. In: Yunus, M., Iqbal, M. (Eds.), *Plant Response to Air Pollution*. Wiley, pp. 167–178.
- King, J.S., Pregitzer, K.S., Zak, D.R., Karnosky, D.F., Isebrands, J.G., Dickson, R.E., Hendrey, G.R., Sober, J., 2001. Fine root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen is affected by elevated CO₂ and tropospheric O₃. *Oecologia* 128, 237–250.
- Kubiske, M.E., Godbold, D.L., 2001. Growth and function of roots and root systems. In: Karnosky, D., Scarascia-Mugnozza, G., Ceulemans, R., Innes, J. (Eds.), *The Impact of Carbon Dioxide*

- and Other Greenhouse Gasses on Forest Ecosystems. CABI Publishing, New York, pp. 325–340.
- Lindroth, R.L., Kinney, K.K., Platz, C.L., 1993. Responses of deciduous trees to elevated atmospheric CO₂: Productivity, phytochemistry and insect performance. *Ecology* 74, 763–777.
- Lindroth, R.L., Roth, S., Kruger, E.L., Volin, J.C., Koss, P.A., 1997. CO₂-mediated changes in aspen chemistry: effects on gypsy moth performance and susceptibility to virus. *Global Change Biol.* 3, 279–289.
- Mankovska, B., Percy, K., Karnosky, D.F., 1998. Impact of ambient tropospheric O₃, CO₂, and particulates on the epicuticular waxes of aspen clones differing in O₃ tolerance. *Ekologia (Bratislava)* 18 (2), 200–210.
- Mankovska, B., Percy, K., Karnosky, D.F., 2003. Impact of greenhouse gases on epicuticular waxes of *Populus tremuloides* Michx: results from an open-air exposure and a natural O₃ gradient. *Ekologia* (in press).
- Mengden, K., 1996. Fungal attachment and penetration. In: Kerstiens, G. (Ed.), *Plant Cuticles*. Bios Scientific Publishers, pp. 175–188.
- Moore, B.D., Cheng, S.H., Sims, D., Seemann, J.R., 1999. The biochemical and molecular basis for photosynthetic acclimation of elevated atmospheric CO₂. *Plant Cell Environ.* 22, 567–582.
- Noormets, A., Sober, A., Pell, E.J., Dickson, R.E., Podila, G.K., Sober, J., Isebrands, J.G., Karnosky, D.F., 2001a. Stomatal and nonstomatal control of photosynthesis in trembling aspen (*Populus tremuloides* Michx.) exposed to elevated CO₂ and O₃. *Plant Cell Environ.* 24, 327–336.
- Noormets, A., McDonald, E.P., Kruger, E.L., Isebrands, J.G., Dickson, R.E., Karnosky, D.F., 2001b. The effect of elevated CO₂ and/or O₃ on potential plant level carbon gain in aspen. *Trees* 15, 262–270.
- Norby, R.J., Wullschlegel, S.D., Gunderson, C.A., Johnson, D.W., Ceulemans, R., 1999. Tree responses to rising CO₂ in field experiments: implications for the future forest. *Plant Cell Environ.* 22, 683–714.
- Percy, K.E., Baker, E.A., 1987. Effects of simulated acid rain on production, morphology and composition of epicuticular wax and on cuticular membrane development. *New Phytol.* 107, 577–589.
- Percy, K.E., Baker, E.A., 1988. Effects of simulated acid rain on leaf wettability, rain retention and uptake of some inorganic ions. *New Phytol.* 108, 75–82.
- Percy, K.E., McQuattie, C.J., Rebbeck, J.A., 1994. Effects of air pollutants on epicuticular wax chemical composition, pp. 67–79. In: Percy, K.E., Cape, J.N., Jagels, R., Simpson, C.M. (Eds.), *Air Pollutants and the Leaf Cuticle*. In: NATO ASI Series, Vol. G-36. Springer-Verlag, Heidelberg.
- Percy, K.E., Awmack, C.S., Lindroth, R.L., Kubiske, M.E., Kopper, B.J., Isebrands, J.G., Pregitzer, K.S., Hendrey, G.R., Dickson, R.E., Zak, D.R., Oksanen, E., Sober, J., Harrington, R., Karnosky, D.F., 2002. Altered performance of forest pests under CO₂- and O₃-enriched atmospheres. *Nature* 420, 403–407.
- Stevenson, D.S., Johnson, C.E., Collins, W.J., Derwent, R.G., Shine, K.P., Edwards, J.M., 1998. Evolution of tropospheric ozone radiative forcing. *Geophys. Res. Lett.* 25, 3819–3822.
- Stott, P.A., Tett, S.F.B., Jones, G.S., Allen, M.R., Mitchell, J.F.B., Jenkins, G.J., 2000. External control of 20th century temperature by natural and anthropogenic forcing. *Science* 290, 2133–2137.
- Tjoelker, M.G., Oleksyn, J., Reich, P.B., 1998. Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO₂ and temperature. *Tree Physiol.* 18, 715–726.
- Tuskan, G.A., Walsh, M.E., 2001. Short-rotation woody crop systems, atmospheric carbon dioxide and carbon management: A U.S. case study. *The Forest Chron.* 77, 259–264.

- Wang, D., Bormann, F.H., Karnosky, D.F., 1986a. Regional tree growth reductions due to ambient ozone: evidence from field experiments. *Environ. Sci. Technol.* 20, 1122–1125.
- Wang, D., Karnosky, D.F., Bormann, F.H., 1986b. Effects of ambient ozone on the productivity of *Populus tremuloides* Michx. grown under field conditions. *Can. J. Forest Res.* 16, 47–55.
- Wustman, B.A., Oksanen, E., Karnosky, D.F., Sober, J., Isebrands, J.G., Hendrey, G.R., Pregitzer, K.S., Podila, G.K., 2001. Effects of elevated CO₂ and O₃ on aspen clones varying in O₃ sensitivity: can CO₂ ameliorate the harmful effects of O₃? *Environ. Pollut.* 115, 473–481.