Environmental Pollution 199 (2015) 119–129

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Ozone affects growth and development of *Pieris brassicae* on the wild host plant *Brassica nigra*

Eliezer Khaling [a, *], Stefano Papazian [b], Erik H. Poelman [c], Jarmo K. Holopainen [a], Benedicte R. Albrectsen [b, d], James D. Blande [a]

[a] *Department of Ecology and Environmental Science, University of Eastern Finland, P.O.Box 1627, FIN-70211, Kuopio, Finland*
[b] *Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, 90187, Umeå, Sweden*
[c] *Laboratory of Entomology, Wageningen University, P.O. Box 8031, NL-6700, EH, Wageningen, The Netherlands*
[d] *Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK 1871, Frederiksberg C, Denmark*

ARTICLE INFO

Article history:
Received 6 September 2014
Received in revised form
22 January 2015
Accepted 25 January 2015
Available online

Keywords:
Brassica nigra
Pieris brassicae
Ozone exposure
Growth and performance
Defence compounds
Phenolics
Glucosinolates

ABSTRACT

When plants are exposed to ozone they exhibit changes in both primary and secondary metabolism, which may affect their interactions with herbivorous insects. Here we investigated the performance and preferences of the specialist herbivore *Pieris brassicae* on the wild plant *Brassica nigra* under elevated ozone conditions. The direct and indirect effects of ozone on the plant-herbivore system were studied. In both cases ozone exposure had a negative effect on *P. brassicae* development. However, in dual-choice tests larvae preferentially consumed plant material previously fumigated with the highest concentration tested, showing a lack of correlation between larval preference and performance on ozone exposed plants. Metabolomic analysis of leaf material subjected to combinations of ozone and herbivore-feeding, and focussing on known defence metabolites, indicated that *P. brassicae* behaviour and performance were associated with ozone-induced alterations to glucosinolate and phenolic pools.

1. Introduction

Global climate change and air pollution are intrinsically linked and represent two of the greatest environmental problems of our time (Unger, 2012). Anthropogenic activities lead to the emission of primary pollutants, including volatile organic compounds (VOCs) and nitrogen oxides (NO_x), which are involved in the atmospheric reactions that yield the secondary pollutant tropospheric ozone (Jenkin and Clemitshaw, 2000). Concentrations of ground-level ozone peak when light levels and temperatures are high, which may be further exacerbated by climate change (The Royal Society, 2008; Fuhrer, 2009). In turn, tropospheric ozone has a positive radiative forcing, which may promote a warmer climate (Unger, 2012).

Importantly, ozone is a phytotoxic gas that negatively affects numerous agricultural and horticultural plants (Booker et al., 2009; Leisner and Ainsworth, 2012; Oksanen et al., 2013), including brassicaceous species (Black et al., 2007; De Bock et al., 2011; Vanderneiren et al., 2012). Ozone enters plants through stomata and degrades in the apoplast forming reactive oxygen species (ROS), which cause oxidative stress and activate several signal transduction pathways involved in stress responses (Kangasjärvi et al., 2005). Ozone stress to plants also results in cell death, decreased photosynthesis, early senescence and altered nutritional quality (Rao and Davis, 2001; Black et al., 2007; Oksanen et al., 2013). In a study of sugar beet and spring rape, ozone exposure caused dose-dependent increases in foliar sucrose levels (Köllner and Krause, 2003), which potentially elevates their nutritive value.

Ozone-induced changes to the nutritional quality of plants has the potential to influence plant-herbivore interactions, indeed empirical evidence supports such a hypothesis (Fluckiger et al., 2002; Valkama et al., 2007). However, the effects on plant-herbivore interactions are variable, with enhancement (Chappelka et al., 1988; Bolsinger et al., 1992), reduction (Coleman and Jones, 1988; Jøndrup et al., 2002) or no effect on (Fortin et al., 1997; Lindroth et al., 1993) herbivore performance for different plant-herbivore combinations. The only previous study to address such questions in a brassicaceous system used ozone sensitive and

---

* Corresponding author.
E-mail address: eliezer.khaling@uef.fi (E. Khaling).

http://dx.doi.org/10.1016/j.envpol.2015.01.019
0269-7491/© 2015 Published by Elsevier Ltd.

resistant varieties of field mustard (*Brassica rapa* L.) and large cabbage white butterfly larvae (*Pieris brassicae*) (Jøndrup et al., 2002). A significant increase in the time to pupation was observed for larvae feeding on ozone-fumigated ozone sensitive plants, but not for the resistant variety. In the same study, 3rd and 4th instar larvae showed no preference for either ozone treated or control leaf discs (Jøndrup et al., 2002).

The effects of ozone on the performance of herbivorous insects are less predictable than the effects of other pollutants such as SO_2, NO_x and pollutant mixtures (Holopainen, 2002; Butler and Trumble, 2008), and do not always correlate with plant nutritional properties. Therefore, it is important to consider the roles of phytochemicals (such as glucosinolates and phenolics) in shaping the performance of herbivorous insects (Bennett and Wallsgrove, 1994). Ozone, like many other abiotic stressors, can influence concentrations of defence-related phytochemicals by inhibiting carbohydrate production or by modulating defence signalling pathways both in the brassicaceae (Gielen et al., 2006; Himanen et al., 2008; Rozpądek et al., 2013) and in other species (Lavola et al., 1994; Saleem et al., 2001; Simmonds, 2003; Peltonen et al., 2006; Vigue and Lindroth, 2010). Glucosinolates are nitrogen and sulphur-containing metabolites characteristic of the Brassicaceae (Halkier and Gershenzon, 2006; Grubb and Abel, 2006; Sønderby et al., 2010) and are, in addition to their volatile breakdown products, involved in biological interactions with herbivores (Bruce, 2014). Glucosinolates, for example, deter many generalist herbivores (Noret et al., 2005; Halkier and Gershenzon, 2006), but may stimulate feeding by specialist herbivores adapted to brassicaceous plants (Renwick and Lopez, 1999; Bridges et al., 2002; Miles et al., 2005; Smallegange et al., 2007). Glucosinolate biosynthesis is modulated in response to both abiotic and biotic stresses including drought (Novak and Haslberger, 2000), salt (Qasim et al., 2003), nitrogen dioxide (Shattuck and Wang, 1993), ozone (Gielen et al., 2006), bacterial (Aires et al., 2011) and fungal (Rostás et al., 2002) pathogens and herbivorous insects (Müller and Sieling, 2006; Rostás et al., 2002). The complex interactions of different stresses often have unpredictable effects on the expression of phytochemicals with implications for plant-herbivore interactions (Dicke et al., 2009). Few studies have addressed the effects of multiple stresses on glucosinolate concentrations, although a recent study showed a near additive effect of simultaneous exposure to an oomycete *Albugo* spp. and a herbivorous flea beetle (*Phyllotreta nemorum*) on glucosinolate concentrations (van Mölken et al., 2014).

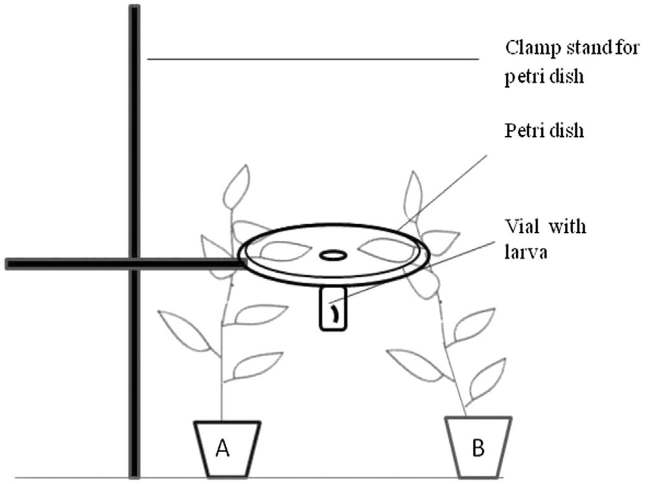

Fig. 1. Set-up for the choice test experiment. Intact plants A and B belong to two different treatments.

Research on the effects of ozone on plant-insect herbivore interactions has mainly focussed on cultivated plant species and forest trees (Lindroth, 2010; Valkama et al., 2007), making it difficult to draw broader ecological conclusions. Artificial selection may alter the cultivated plants' responses to various abiotic and biotic stresses, and specific expression of stress-mediated changes may vary between cultivated plants and their wild counterparts (Gols et al., 2008). Many of the earlier studies have focussed on the indirect effects of ozone exposure, largely utilizing cut leaves or leaf discs. Here we investigated a system comprising the specialist *Brassica*-feeding Lepidopteran *Pieris brassicae* and its wild host *Brassica nigra*. We tested the direct effects of ozone on the growth and development of *P. brassicae* larvae by exposing the whole plant-herbivore system to different ozone levels and the indirect effects through oxidative stress to the host plant only. We also tested the indirect effects of ozone on *P. brassicae* preference, using intact plants in all tests. Furthermore, metabolomic analysis of the leaves exposed to ozone and herbivore-feeding was performed to evaluate the induction of defence responses that may be linked to herbivore-performance and preference.

2. Methods and materials

2.1. Plants and insects

B. nigra L. (Black mustard) seeds were harvested from wild populations near Wageningen University, The Netherlands. They were planted individually in 1 L plastic pots filled with a 3:1 mix of peat and sand and grown under greenhouse conditions (light/dark cycle: 18 h/6 h; daytime temperature 23 °C, light intensity 286μmol m^{-2} s^{-1} and relative humidity (RH %) 60%; night temperature 18 °C and RH 80%) at the University of Eastern Finland, Kuopio, Finland. The seedlings were fertilized with 0.1% 5-Superex (N:P:K 19:5:20), Kekkilä, Finland. Eggs of *Pieris brassicae* (Lepidoptera: Pieridae) came from cultures at the Laboratory of Entomology, Wageningen University, where they were maintained on Brussels sprout plants (*Brassica oleracea* var *gemmifera* L. cultivar Cyrus) in a climate controlled room of temperature 20 ± 2 °C, RH 50–70%. *P. brassicae* were not exposed to ozone-fumigated plants prior to the experiments.

2.2. Ozone fumigation

Four-week old *B. nigra* plants were moved to growth chambers (Weiss Bio 1300; Weiss Umwelttechnik Gmbh, Preskirchen-Lindenstruth, Germany) and maintained there for five consecutive days under conditions of temperature: 23 ± 3 °C, RH: ~60%, photoperiod L16h:D8h and light intensity: 300μmol m^{-2} s^{-1}. The chambers are adapted to have independently regulated ozone concentrations, which were set at ambient, 70 ppb, or 120 ppb. Ambient ozone concentrations fluctuated between 15 and 20 ppb, the other chambers had elevated concentrations from 04:00 to 20:00 and a basal concentration of 30 ppb from 20:00 to 04:00. This approximated natural diurnal variation in ozone concentration. The plants were watered daily. Introduction of plants into the chambers was staggered so that there was a constant turnover of five-day exposed plants. The plants did not develop flowers through duration of experiments.

2.3. Growth response experiments

2.3.1. Whole system effect of ozone

First instar *P. brassicae* were weighed individually using a microbalance (Mx5, Meter Toledo, Switzerland) and transferred to pre-fumigated *B. nigra* plants with a fine brush sterilized with 70%