Causes of leaf area reduction and implications of acclimation to UV-B radiation in Pisum sativum L

Bolink, Esther

Summary

The amount of Ultraviolet-B (UV-B) radiation reaching the Earth's surface has gradually increased in the last 30 years, due to human society induced atmospherical pollution (Chapter 1). Chlorofluorocarbons (CFC's), which have been used as refrigerants, aerosol propellants and solvents in the electronic industry bring about decreases in stratospheric ozone. Since the ozone layer absorbs UV radiation at wavelengths below 330 nm, a decrease in stratospheric ozone leads to an increase in UV-B radiation reaching the Earth. Although the international community tries to stop the pollution of the atmosphere with ozone destroying products, as agreed in the Montreal Protocol (1987), it is predicted that the thinning of the ozone layer will continue until at least 2010, due to the greenhouse effect, which indirectly increases the destruction of ozone.

UV-B radiation can be harmful to living organisms, since it may damage DNA, RNA, proteins and membranes. In plants, increased UV-B radiation causes changes in photosynthesis, growth, development, morphology and production of various secondary metabolites. In the research described in this thesis studies were conducted with pea plants (*Pisum sativum* L. mutant *Argenteum*) in growth chambers. The aim of the study was to investigate the mechanisms underlying the inhibition of leaf growth by increased UV-B radiation in pea plants and the physiological implications of the UV-B induced increase in secondary metabolites.

In Chapter 2 pea plants were studied which were either exposed to two levels of supplemental UV-B radiation, supplemental UV-A radiation, or no supplemental radiation. UV-B radiation significantly reduced shoot length, total leaf area and SLA, and these reductions increased with UV-B intensity. After about 14 days a small delay in plant development became evident in the high UV-B treated plants. This delay increased with time and was related to a reduction in plastochron index, which was significant after 22 days.

Leaf expansion was studied at successive stages of plant development, by regular measurement of leaflet area of leaves 1 to 6, from the time these leaves emerged from the stipules until full expansion (Chapter 2). In leaves 2 to 6 UV-B treatment significantly reduced final leaf area, but growth duration was not affected. The reduction of leaf area by exposure to UV-B was more pronounced in every subsequent leaf, and thus increased with exposure time. Microscopic measurements of epidermal cell size and cell number showed that in earlier developed 3rd leaves cell number was significantly decreased by UV-B, but in leaves developed later (5th, 6th), reduction of cell size was mainly responsible for the reduced leaf area in UV-B treated plants. Apparently, the mechanism of UV-B induced growth reduction of pea leaves depended on the developmental stage of the plant.

Since UV-B radiation decreased cell size in the 5th and 6th leaf of pea plants, the cause of this decrease was investigated. Cell area reduction can be caused by reduced wall extensibility or reduced turgor. In Chapter 3 it was shown that turgor pressure was not affected by UV-B radiation. However, cell wall extensibility in strips of young expanding leaves was significantly reduced in UV-B treated plants. A common response of plants to enhanced UV-B radiation is an increase in phenolic compounds, and increased phenolic crosslinking could cause decreased wall extensibility. Therefore, the activity of wall-bound peroxide, the enzyme catalysing the crosslinking reaction, was studied (Chapter 3). No changes in wall-bound peroxidase activity were found after UV-B treatment. It was suggested that a decrease in wall extensibility in young, expanding leaves contributed to the observed leaf area reduction in UV-B treated pea plants, but that this decrease was not related with changes in peroxidase activity.

In Chapter 4 apoplastic acidification in leaf epidermal strips in response to visible light was measured in plants supplemented with UV-B radiation and control plants. Acidification of the cell wall is important for cell expansion, and disturbance of this process could possibly reduce cell expansion, resulting in a decreased leaf area. In several experiments the acidification response of leaf epidermal strips of UV-B irradiated plants

seemed to be reduced compared to controls, but this decrease turned out to be not statistically significant, due to high variances in acidification responses between and within individual leaf strips.

Chapter 5 focussed on possible implications of increased phenolic compounds, mainly flavonoids, in plants exposed to supplemental UV-B radiation. The flavonoids kaempferol and quercitin, which are strongly induced by UV-B radiation in pea, are inhibitors of polar auxin transport, which can be a direct cause of reduced plant length. UV-B radiation reduced internode length and simultaneously increased UV-B absorbing capacity (caused by an increase in flavonoids) in stem sections (Chapter 5). However, polar auxin transport rates, studied with radioactive labelled [^{3}H]IAA, did not significantly differ in stem sections of UV-B treated and control plants. Differences in location of the UV-B induced flavonoids and of polar auxin transport might explain the absence of a causal relationship between polar auxin transport and UV-B induced inhibition of elongation growth.

In Chapter 6 an increased tolerance to high light (HL) stress (PAR: 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was found in pea and bean (*Phaseolus vulgaris* L.) plants after exposure to enhanced levels of UV-B radiation. A significantly slower decline of maximum quantum efficiency of PSII (F_v/F_m), together with a slower decline of oxygen evolution during HL stress was observed in leaf discs of UV-B treated plants compared to controls in both plant species. In pea leaves the total pool of xanthophyll cycle pigments was increased by UV-B radiation, and in bean leaves thiol concentrations were significantly enhanced. These pigments, together with increases leaf thickness, probably protected the plants against photoinhibition induced by the HL intensity commonly accompanying conditions of high UV-B radiation.

In conclusion, the experiments described in the present thesis show that UV-B radiation affects different mechanisms controlling leaf growth in pea plants (Chapter 7). It depends on the stage of leaf and plant development which mechanism will contribute most to growth reduction. Early in plant development cell division is reduced by UV-B radiation, causing smaller leaves, while later in development cell expansion is

reduced. Although UV-B radiation increased levels of flavonoids which are known inhibitors of polar auxin transport, this transport process was not decreased in UV-B treated plants and could therefore not have caused decreased expansion growth. UV-B radiation did cause a significant reduction in cell wall extensibility, which may be (partly) responsible for the reduction in cell expansion. It was shown that levels of cell wall bound peroxidase were not affected by UV-B radiation, so the decrease in wall extensibility was probably not caused by increased phenolic crosslinking. Further research is necessary to elucidate which enzymes are involved in the reduction of wall extensibility by UV-B radiation and to what extend flavonoids and auxin levels are implicated in UV-B induced reduction of leaf growth.