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Changes in Leaf Water Potential, Lethal Temperature and Carbohydrate Content of Wintergreen (*Pyrola japonica* Klenze) during Overwintering

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越冬 중 노루발의 水分포텐셜, 致死溫度 및 炭水化物量의 變化

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ABSTRACT

Changes in water potential, lethal temperature and carbohydrate content in the leaves of wintergreen (*Pyrola japonica*) during overwintering were investigated. Leaf water potenttial was kept at −2 bars in the tender stage before October, decreased to −46 bars in the dormancy stage and increased to −2 bars again after dehardening. Lethal temperatures of the leaf tissue were −7°C in the tender stage and −19°C in the dormancy stage, but did not recover up to that of the tender stage during dehardening. Peak of soluble sugar content coincided with the nadir of the leaf water potential. There were close relationships among daily minimum air-temperature, leaf water potential and lethal temperature in changing patterns during overwintering.

INTRODUCTION

Wintergreen (*Pyrola japonica* Klenze) is an evergreen perennial herb growing under deciduous forests throughout Korean peninsula. Plant of this species overwinters with evergreen broad leaves in spite of low temperature occasionally falling to −30°C.

Before plants acquire cold resistance, the process of cold hardening has to be experienced during autumn. Cold hardening necessitates both a low temperature and short day length as the inducing factors (Burke *et al.*, 1976; Chen *et al.*, 1977; Parsons, 1978). Several physiological factors such as soluble sugar concentration and water potential are expected to change with relation to an increase of cold resistance (Fuchigami *et al.*, 1971).

This study is to investigate the changes in water potential, lethal temperature and carbohydrates of the wintergreen leaves, and to elucidate their relationship in changing patterns during overwintering.

MATERIAL AND HABITAT

Wintergreen (*Pyrola japonica*) is broad-leaved evergreen herb surviving during winter in Korean peninsula. Plant materials used in the present study were sampled in *Quercus mongolica* forest, associated with such shrubs as *Rhododendron mucronulatum* and *R. schlippenbachii*, on the southeast slope of Mt. Dobongsan near Seoul, Korea (Fig. 1).

The rhizome of the plant extends laterally into a humus-rich soil (A_{00} layer) covered with the duff. Due to the dense canopy and thick duff, soil moisture was maintained approximately at 15% on the dry weight basis during study period. Radiation received by the wintergreen leaves, however, might be restricted by the dense canopy through growing season except in early spring.

Daily maximum and minimum air-temperatures at 30 cm above ground and daily minimum soil-temperature at 10 cm below the ground in the habitat were respectively −3.0°C, −14.5°C and −2.5°C for study period and snow covered on the ground from December 28, 1984 to February 10, 1985 (Fig. 2).

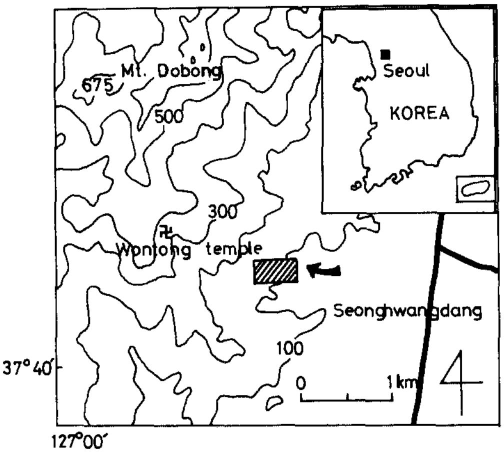

Fig. 1. Map showing the sampling site.

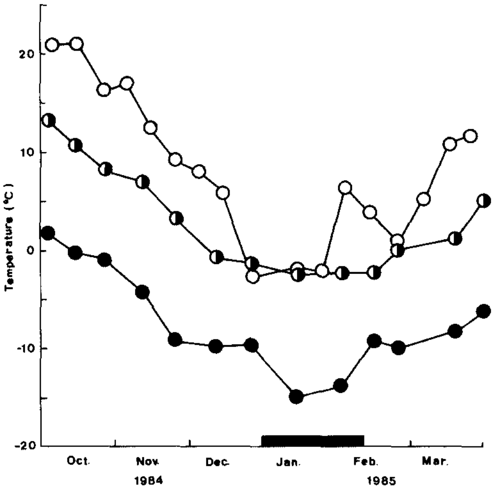

Fig. 2. Daily maximum air-(○), minimum air-(●) and minimum soil-temperatures (◑) and snow cover (black) in the habitat for study period from October, 1984 to March, 1985.

METHODS

To measure leaf water potential the wintergreen plants were dug out, put into polyethylene bag and then brought to laboratory. As soon as possible, water potential of the leaves was determined by Shardakov method (Ross, 1974).

To determine lethal temperature of the leaf tissue as well as othe organs, the tissues were frozen as follows. The materials were wrapped with aluminum foil, put in the −3°C refrigerator for 1 hr and then frozen gradually at the interval of 5°C per hour from −3°C to −30°C, using several refrigerators. The materials were kept in a small ice box to avoid freezing shock at each temperature interval. Subsample was taken out at each interval along the freezing gradient, warmed gradually to room temperature and then placed at 20°C for 24 hr. Grades of cold injury were marked in terms of color according to the method of Van Huystee *et al.* (1967); green color over whole area gave 0% injury, dark brown over whole area gave 100% and intermediate area of green or dark brown gave corresponding % injury. Lethal temperature of the material was designated as grade of 50% injury or LT_{50}.

To determine content of soluble sugars and starch, the leaves sampled were dried at 60°C for 27 hr, pounded finely up and then kept in a 3°C desiccator until analysis. Analyses were made after Anthrone method (Hansen and Moller, 1975).

RESULTS

Lethal temperature and phenology

Lethal temperatures of the wintergreen were −18 to −19°C for leaf blade and petriole, −27°C for bud and −9 to −13°C for stalk and rhizome in January, 1985 (Fig. 3). These data indicate the maximum cold resistance of different plant organs achieved during the cold hardening. Lethal temperature of the leaf blade was −7°C in October, an initial stage of cold acclimation, and decreased into −19°C in the end of December, a final stage of cold acclimation (Fig. 4). Thereafter the plant was gradually dehardened. Daily minimum air-temperature recorded in January was −14.5°C, which is above lethal temperature of the plant (Fig. 2). Lethal temperature of this dehardened stage, however, remained −15 to −16°C, which was below than the level of the tender stage in past October in spite of full recovery of leaf water potential as descirbed later. Snow covering on the plant from December to February might aid survival of the wintergreen during cold winter. The wintergreen plant began shooting up as early as the end of February when snow still remained on the ground, grew up rapidly and completed the full growth the early spring before leafing of the tree canopy. This strategy could protect the plant from casual drop of temperature in the early spring. The perennial strategy of the wintergreen under tree canopy showed adaptation for both the winter cold stress and the radiation deficiency during growing season.

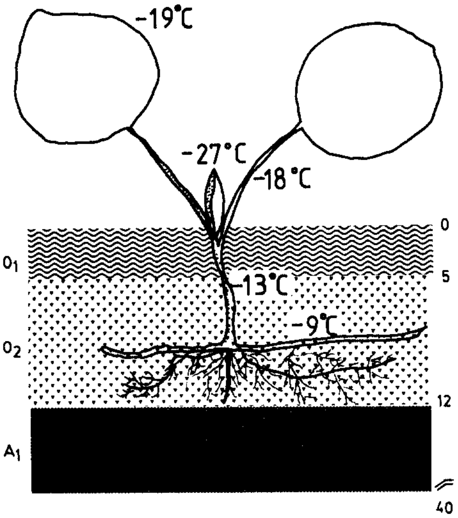

Fig. 3. LT_{50} in various organs of the wintergreen under cold resistance in January, 1985.

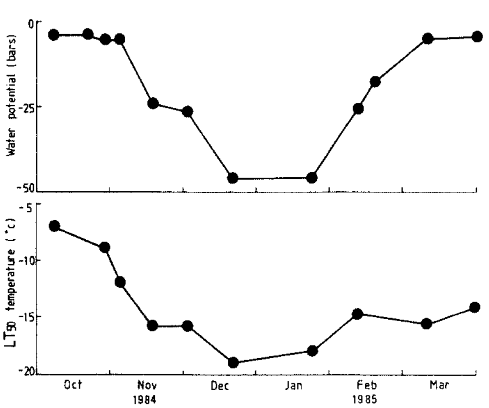

Fig. 4. Changes of water potential (above) and LT_{50} (below) in wintergreen leaves during overwintering.

Leaf water potential

Although leaf water potential of the wintergreen was kept at as high as -2 bars in the tender stage before October, it decreased gradually during cold hardening (Fig. 4). Overwintering is divided into three periods depending on lethal temperature change: cold hardening, dormancy and dehardening stages (Larcher, 1980). The leaf water potential decreased proportionally to decreasing daily minimum air-temperature during the hardening stage from the end of October to the end of December. The leaf water potential was as low as -46 bars in the dormancy stage from the end of December to the end of January. In this stage the leaves showed extremely stiff, desiccated and frozen appearance. The leaf water potential in the dehardening stage increased gradually to -2 bars again before the beginning of March.

Under the similar climate conditions, the leaf water potentials of evergreen conifers in the dormancy stage were -23 bars for spruce (*Picea jezoensis*) and yew (*Taxus cuspidata*), and -12 bars for *Pinus* spp. (Kim and Lee, 1987). The leaf water potential of the wintergreen was twice as low as the former two conifers.

Changes of carbohydrate content

The content of soluble sugars in the leaves increased from October to mid-December and decreased thereafter, whereas the content of starch decreased to mid-November and increased thereafter (Fig. 5). These results indicate that starch is hydrolized to low molecular sugars during cold hardening (Sakai and Yoshida, 1968; Levitt, 1972; Dear, 1973; Li and Palta, 1978; Kaurin *et al.*, 1981). It was found that cold induced an increase in soluble sugars and a decrease in starch, with disappearance of starch from chloroplasts after cold hardening (Chen *et al.*, 1977).

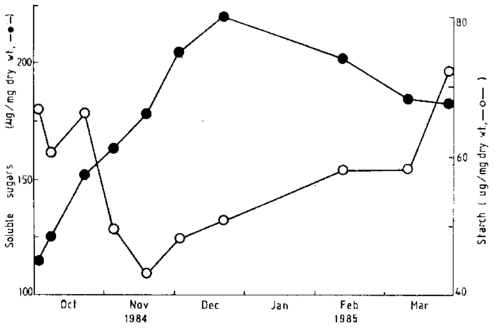

Fig. 5. Changes of concentrations of starch and soluble sugars in wintergreen leaves during overwintering.

DISCUSSION

Foregoing data are analyzed on relationships among the environmental and physiological factors (Fig. 6). The leaf water potential decreased proportionally to the minimum air-temperature at lower than -4°C which may be a transit point for cold hardening, except for data of dehardening, and it had already reached to the lowest level before air temperature dropped to the lowest, and also remained during the coldest period (Fig. 6a). The nadir of leaf water potential of the wintergreen during overwintering might subject protoplast of the plant cell to heavy drought stress (Burke *et al.*, 1976). It was suggested, therefore, that cold resistance might be closely related to drought resistance in order to survive in harsh winters (Li and Weiser, 1971; Mckenzie *et al.*, 1974; Chen *et al.*, 1977; Kim and Lee, 1987). The lethal temperature of the wintergreen leaves decreased logarithmically as daily minimum air-temperature decreased (Fig. 6b). This indicates that the leaves are sensitive to air-temperature in the initial stage of the hardening but not so in the dormancy stage. Similar trend in sensitivity to air-temperature was observed in *Salix* sp. (Sakai, 1970).

The lethal temperature of the leaves suddenly changed with a range of −7 to −12°C at the early hardening stage with high water potential but gradually and proportionally changed as water potential decreased at the late hardening and dormancy stages (Fig. 6c). The lethal temperature recovered in the dehardening stage was considerably lower than that in the early hardening stage (van Huystee *et al.*, 1967).

Soluble carbohydrates are one of the factors most considerably changing during cold hardening (Levitt, 1980), and may play a role of protecting cell membrane from freezing injury (Santarius, 1973; Li, 1985). The highest content of the soluble sugars corresponded the lowest level of leaf water potential at the end of December (Figs. 4 and 5). The changing patterns of the two.

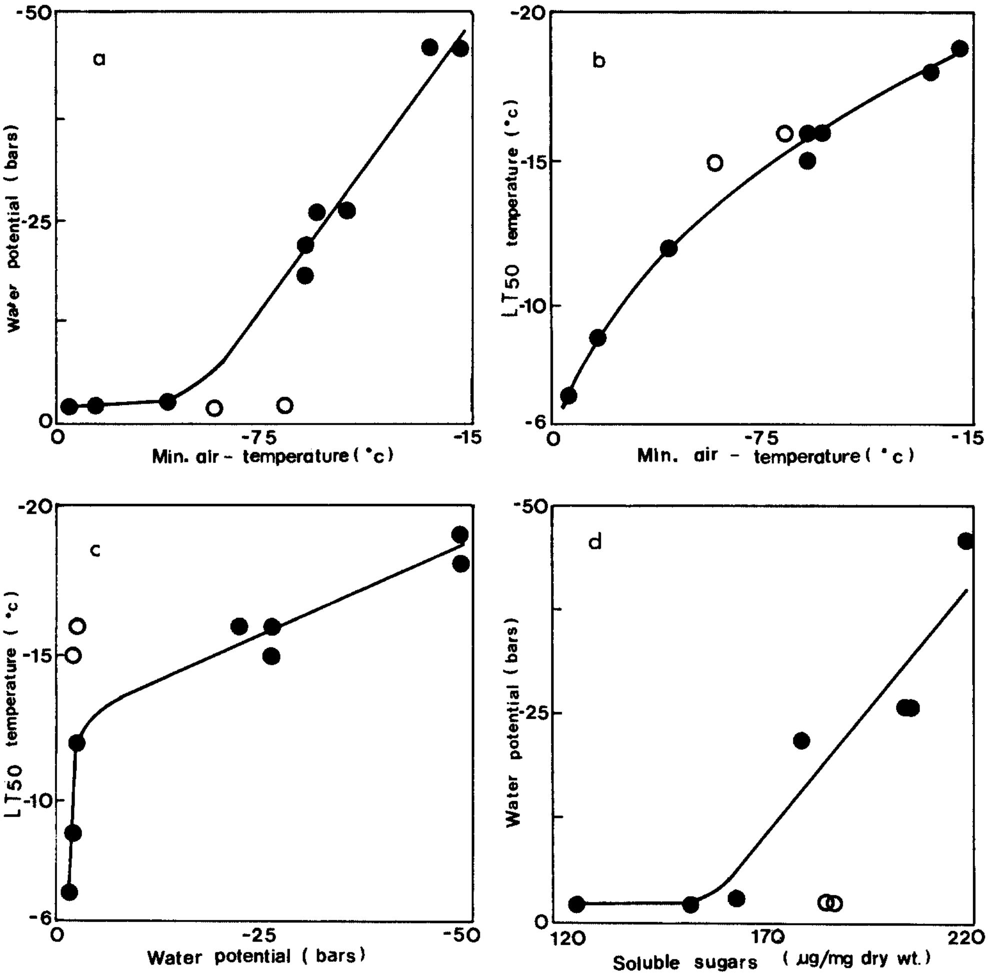

Fig. 6. Relationships between the daily minimum air-temperature and water potential (a), and LT_{50} (b), between water potential and LT_{50} (c) and soluble sugar content (d) in wintergreen leaves during overwintering. Closed and open circles indicate data obtained in hardening and dehardening stages.

factors showed considerably close relationship during overwintering with more than 170 μg/mg dry wt. of soluble sugars and lower than −4 bars of water potential (Fig. 6d). Water potential can decrease not only by the increase of solutes, but also by the loss of water from leaf tissues (Chen *et al.*, 1977). It was reported that plants induce the rapid loss of water by decreasing the stomatal resistance of leaf and increasing the transpiration (Parsons, 1978).

Beginning of dormancy stage for the wintergreen leaves coincided with both the periods of the nadir in the lethal temperatures and of the highest soluble sugars in the end of December, which was earlier 15 days than the period of the lowest temperature in daily minimum air-temperature (Fig. 7).

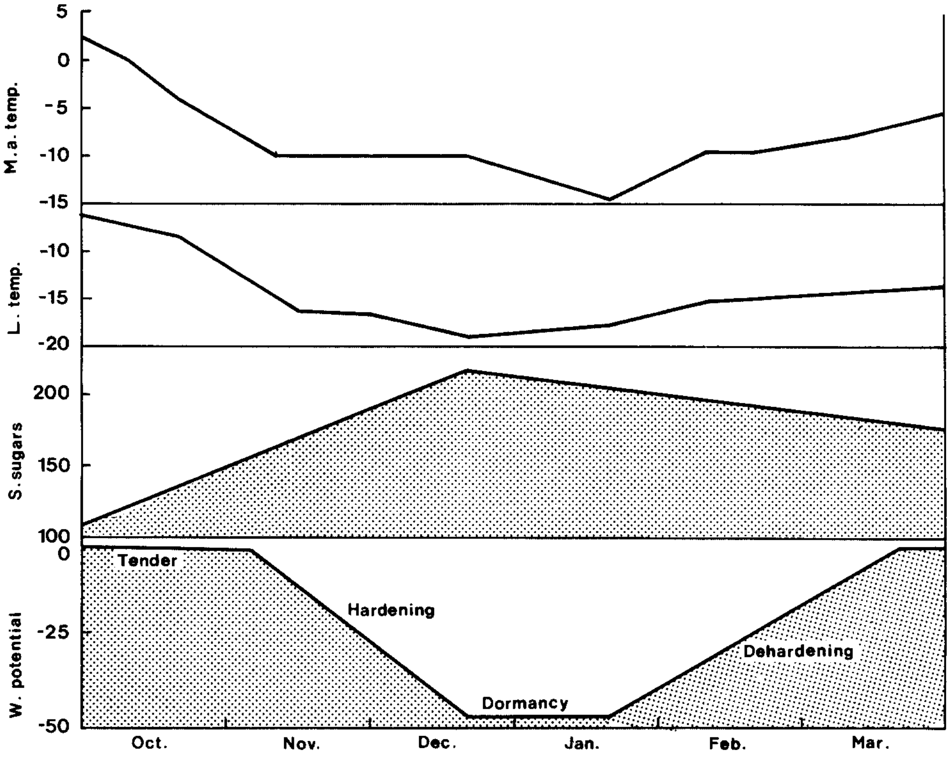

Fig. 7. Diagram showing physiological changes of the wintergreen leaves related to change of daily minimum air-temperature during overwintering.

摘要

도봉산(서울근교)의 신갈나무숲에서 생육하는 越冬과정의 노루발(*Pyrola japonica*) 잎의 水分포텐셜, 生存溫度 및 糖함량의 변화를 측정한 결과로부터 식물의 硬化, 休眠 및 脫硬化를 해석하였다. 수분포텐셜은 11월초순까지 -2bars였고, 12월하순에 -46bars로 저

하하여 1월하순까지 계속된 후 3월초순에 -2 bars로 회복하였다. 생존온도는 10월하순까지 -7℃이었고 12월하순에 -19℃로 낮아짐으로써 일최저기온의 경향과 유사하였다. 可溶性糖은 10월초순~12월하순 사이에 증가하고 그 후 감소하였다. 노루발의 硬化기간은 수분포텐셜과 생존온도의 저하 및 가용성당이 증가하는 기간과 일치하였지만 休眠기간의 시작은 일최저기온이 나타나는 때보다 15일 빨랐으며, 脫硬化기간은 水分포텐셜의 증가 및 가용성당이 감소하는 기간과 일치하였지만 생존온도가 회복하는 시기와는 일치하지 않았다.

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