

Differential Sensitivity to Waterlogging and Cyanogenesis by Peach, Apricot, and Plum Roots¹

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Abstract. Individual plants varied considerably, but peach and apricot were more sensitive to waterlogging than was plum. No differences were established between peach and apricot. All 3 species became more sensitive as temperature was increased between 17 and 27°C. More than half of the plum seedlings survived at 17°C whereas all plants of the other 2 species died. A scion of a more tolerant species did not overcome the sensitivity of the roots.

Both cyanogenic glycoside content and the proportion of it that was hydrolyzed during waterlogging were higher in peach than in plum roots. Exposure of detached root systems of all 3 species to anaerobic conditions caused HCN to be released. The rate of cyanogenesis increased with both temperature and time. Peach and apricot roots were alike in HCN evolution whereas plum roots were lower, with release of HCN being barely detectable at 22°C. Cyanogenesis was significant in peach and apricot at as low as 17°C.

A close association exists among differential sensitivity, glycoside hydrolysis, and cyanogenesis in the absence of O₂. However, the latter may be secondary, though contributory, to cellular disorganization as a cause of sensitivity.

Fruit tree species possess a wide range of tolerance to long periods in water-saturated soils (waterlogging) (2, 6, 13). Peach, apricot and almond roots are generally considered highly sensitive, whereas apple, pear, and quince are relatively tolerant. 'Myrobalan' plum is of intermediate tolerance (6). Except with citrus (14) and avocado (15), practically all reports on tolerance to waterlogging are based on field observations, which, at best, can only be regarded as subjective.

Excessive water in the soil leads to reduced O₂ levels, both from displacement of soil air and the low solubility and rate of diffusion of O₂ in water (4). Some plant species can withstand these conditions better than others, and the tolerance of a given species is temperature-dependent. Apple seedlings supplied with 5% O₂ in a nutrient solution made some root growth, whereas peach and 'Myrobalan' plum required more than 15% O₂ (3). Stolzy and Letey (13) reviewed correlations of adverse plant responses with low O₂ diffusion rates in unsaturated soils.

Since soil types and topography of many areas prevented adequate drainage, rootstocks are needed which are tolerant to such conditions. Means of rapid measurement and an understanding of the primary causes of sensitivity would contribute greatly to the development of tolerant rootstocks. Experiments were conducted to accurately measure the comparative tolerance of peach, apricot, and plum to waterlogged conditions as influenced by temperature. Because these 3 species contain a cyanogenic glycoside, the possible contribution of cyanogenesis to sensitivity was also investigated.

Materials and Methods

Seedlings of peach (*Prunus persica* L., 'Lovell'), apricot (*Prunus armeniaca* L., 'Royal'), and plum (*Prunus cerasifera* Ehrh., 'Myrobalan 3 J') were grown in sterilized sand or in a mixture of sand and peat moss in pots in the greenhouse. All plants were fertilized regularly with Hoagland's solution (8).

For waterlogging treatments, 5-inch pots containing 6-month-old seedlings of each species were placed in 6.25-inch plastic pots, with polyethylene liners separating the 2 containers. Tap water was added to the inner pots to a level of 2 inches above the surface of the medium. The surface of the water, left exposed to the atmosphere, was maintained at this level throughout the experiment. The pots were then placed in controlled-temperature baths at 27, 22, and 17°C in the

greenhouse. Holes were left open in the outer pots to allow circulation of the bath water around the outside of the polyethylene liner. The water of the temperature bath did not come into direct contact with that bathing the plant roots. The time when symptoms of wilting first appeared was recorded.

A number of reciprocal grafts of peach and plum were made when the seedlings were 3 months old. Plants of peach grafted on peach were also developed. The unions were 3 inches above the surface of the medium. Grafted plants were subjected to waterlogging in a controlled-environment room at 27°C. Tap water was maintained in the pot as described above. Fluorescent lighting was supplied on a schedule of 12 hr of light and 12 hr darkness. Intensity of illumination was 300 ft-c 12 inches above the tops of the plants. Relative humidity was maintained at approximately 90%. Untreated plants grew normally under these conditions.

For determination of cyanogenic glycoside content, roots were gently washed and the intact seedling allowed to dry at room temperature for 2 days. The root systems were then severed at the cotyledonary scar and dried further under vacuum for 48 hr at 75°C. The dried tissue was ground in a Wiley mill to pass through a 40-mesh sieve. Approximately 5 mg of powder was weighed into the outside well of a 3-inch Conway microdiffusion dish. To the center well was added 2 ml of 0.1 N KOH. Three milliliters of an emulsin solution (1 mg/ml) was added to the powder, the tops were secured and the enzyme and powder were mixed by gentle rocking. After 3 hr at room temperature, adequate for complete hydrolysis, 1 ml of 10% H₂SO₄ was quickly added to the reaction mixture and the dish was sealed again. An additional 3 hr was allowed for diffusion of HCN into the KOH. One-milliliter aliquots were removed from the KOH center well, diluted with 2 ml of distilled water, and their CN⁻ content was determined by the method of Robbie (11).

For experiments with detached roots, seedlings were removed from pots and their roots placed in tap water. The root systems were cut from the stems just above the first lateral roots. Two root systems were placed in each 150-cc capacity Warburg flask. To each flask was added 4 ml of distilled water, just sufficient to cover the bottom of the flask without covering the roots. A 10-ml beaker containing 2 ml of 0.1 N KOH was placed in the flask to trap HCN. The flasks were attached to Warburg manometers, sealed, and allowed to stand at room temperature for 4 hr of pretreatment. During pretreatment the HCN evolved because of wounding gradually diminished, approaching 0.

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After pretreatment, fresh KOH traps were placed in the flasks, which were then attached to manometers and the roots subjected to modified atmospheres. Gas mixtures were prepared using flowmeters calibrated to deliver known rates of flow for each gas (5). Modified atmospheres were established in the flasks by flushing at the rate of 300 cc/min for 5 min. N₂ gas was passed through a concentrated solution of pyrogallol to remove contaminating O₂.

After flushing, the flasks were transferred to the water bath at the desired temperature. After incubation for various periods the KOH traps were removed and CN⁻ determined as above. When KOH traps were removed at intervals, they were replaced by ones with fresh KOH, and the modified atmospheres re-established. After treatment the roots were dried under vacuum for 48 hr at 45°C and weighed.

Results and Discussion

Differences in the survival of peach, apricot, and plum seedlings after their root environments were saturated with water at 3 temperatures are shown in Fig. 1. The index of plant death was wilting of the leaves. In all cases, plants which wilted failed to recover when the pots were immediately drained. At 27°C, the respective average times for peach and apricot plants to wilt were 5.2 and 5.1 days. The plum seedlings averaged 11.3 days. At 22°C, the respective average times of apricot and peach deaths were 6.8 and 8.5 days. The 1st plum seedling wilted on the 6th day, and the 14th wilted on the 25th day; 2 were still alive after 47 days. At 17°C the difference between peach and apricot, compared with plum, became more apparent. The respective average time at which peach and apricot seedlings died was 12.8 and 10.9 days, whereas only 5 of 16 plum seedlings had wilted by the 47th day.

It has been suggested that the tolerance of species to waterlogging might be due to diffusion of O₂ from the aerial parts of plants to the roots (2, 12). To explore the possibility of a scion influence on sensitivity, reciprocally grafted plum and peach plants were waterlogged at 27°C in a controlled-environment room. Nine plants of each combination were used, in addition to 9 plants with peach scions grafted on peach roots. Treatments were made 6 months after grafting. Plants with peach roots and plum scions required an average of 11 days to wilt, compared with 10 days for peach on peach roots. When peach scions were grafted on plum roots, however, the tolerance to waterlogging increased appreciably, since only 2 of 9 plants wilted by the 14th day. Thus, the site of sensitivity was the roots with no apparent influence from the scion. These results do not completely discredit the theory that diffusion of O₂ from the top of the plant to its roots might account for survival of plum. If it does occur, however, it was not sufficient to affect the sensitivity of peach roots.

The different response of plum with decreased temperature suggests that this species may possess a mechanism for survival that does not exist in peach and apricot, at least at the temperatures imposed. It seems more likely, however, that the phenomena leading to root death are greatly reduced or do not occur at the lower temperatures. Because roots of the 3 species contain a cyanogenic glycoside which, upon hydrolysis, yields HCN, the influence of waterlogging on the levels of this glycoside was measured at 27°C. Ten plants were used in each treatment or control and the results of analyses of individual plants were averaged. The CN⁻ content of roots of peach seedlings which were not waterlogged was 0.134 μmoles/mg dry weight. The amount of glycoside is expressed as CN⁻ since 1 molecule of glycoside yields 1 molecule of HCN upon

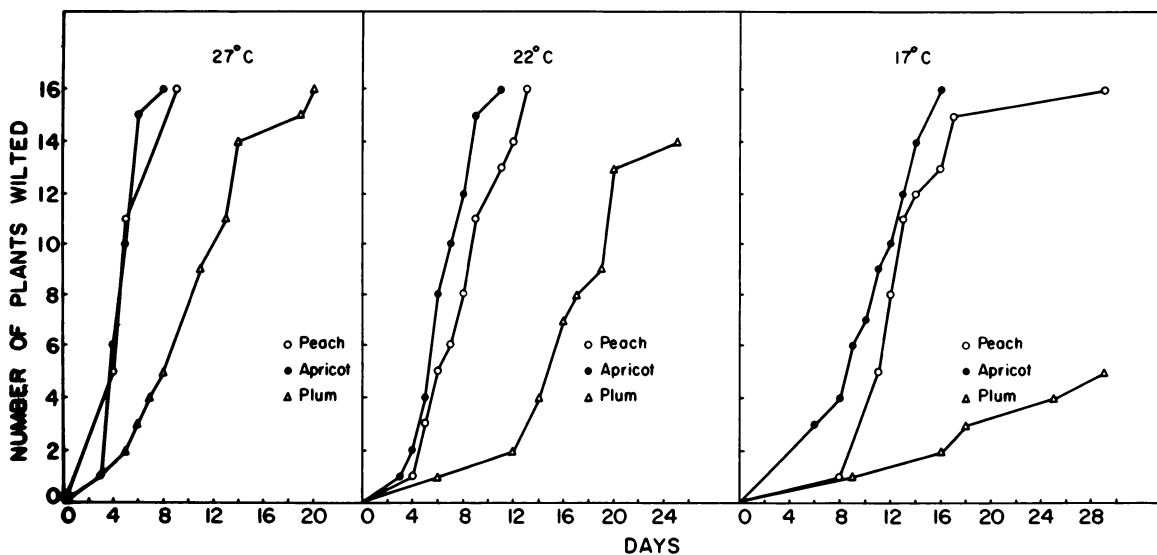


Fig. 1. The effect of waterlogging at different root temperatures on survival of seedlings of peach, apricot, and plum.

Although there was a wide range of sensitivity among individual seedlings of each species, plum was more tolerant to root environments saturated with water than either peach or apricot at all temperatures. There was some indication that apricot might be more sensitive than peach at the 2 lower temperatures, but more seedlings would require testing to establish this assumption. All species decreased in sensitivity when temperature was decreased from 27 to 17°C. Plum seedlings had a greater change in sensitivity over this range than did the other species. Results were similar in an identical experiment using 3 plants of each species. The results confirm field observations regarding differences among peach, apricot, and plum in sensitivity to waterlogging. A similar relationship between temperature and sensitivity exists with a number of other species (4).

Plants which were waterlogged were removed for analysis when they showed the 1st symptoms of wilting. All peach seedlings wilted by the 10th day of treatment. Waterlogging caused a 73% reduction in CN⁻ content of roots to 0.036 μmoles/mg. With plum, 2 controls were included to establish whether temperature alone could affect glycoside levels. The CN⁻ content of roots before waterlogging and of those held at 27°C for 10 days without waterlogging was respectively 0.094 and 0.096 μmoles/mg dry weight. Results were the same in a similar comparison employing 6 peach plants in each treatment. During 10 days of waterlogging the CN⁻ content of plum roots decreased by 37% to 0.060 μmoles/mg. The only plum that wilted did so on the 3rd day, and the glycoside content of its roots was 18% of that of the controls. With both peach and plum, the decreases in cyanogenic

glycoside during waterlogging were highly significant as determined by Student's t-test. Thus, both species show a close association between glycoside hydrolysis and loss of root function.

The cyanogenic glycoside content of untreated roots of plum was 71% of that of peach. However, peach roots lost about 3 times as much glycoside as did plum roots. Thus, the conditions imposed by waterlogging stimulated hydrolysis more in peach than in plum. The HCN released might be expected to have an inhibitory effect on the respiratory metabolism of the root and account for, or contribute to, the greater sensitivity of peach. Excepting 1 plant, the amount of HCN apparently produced by plum during waterlogging was insufficient to affect sensitivity.

Since both the amount and rate of supply of O₂ are greatly reduced during waterlogging (2, 4, 13), the influence of anaerobic conditions on HCN production was determined at several temperatures using detached root systems. When root systems were cut from plants, HCN was evolved. Therefore, a pretreatment of 4 hr in air was employed before modified atmospheres were imposed. During pretreatment, HCN evolution either stopped or declined to very low rates. The rates of HCN subsequently evolved were corrected for rates of production, if any, at the end of pretreatment.

The rates of cyanogenesis by detached root systems of 1-month-old peach, apricot and plum seedlings during 8 hr exposure to 100% N₂ at different temperatures are shown in Fig. 2. Each determination was made in triplicate. With all 3 species, HCN evolution was fastest at 27°C. As the temperatures decreased, the rates of cyanogenesis also declined. Practically no HCN was released from plum roots at 22°C, whereas both apricot and peach produced some HCN at 17°C.

The data were analyzed by Student's t-test, and differences are noted on the basis of significance at the 5% level. HCN evolution by peach at 22 and 27°C was not different from that of apricot. For plum, however, the rates were lower than for the other species at these 2 temperatures. The rates of HCN production by peach at 27, 24.5, 22 and 17°C were

significantly different from each other. With apricot, the rates at 27, 22 and 17°C were different. The difference in rates at 24.5 and 19.5°C was also significant. The 2 rates of HCN released by the plum roots were different from each other.

The rate of evolution of HCN increased with time in N₂ with all 3 species. Approximately 3 times as much HCN was released in the second 4 hr of anaerobiosis than in the first 4 hr. In another experiment, HCN evolution from peach and plum roots in 100% N₂ at 27°C was measured at 2-hr intervals for 25 hr. HCN production by peach roots was 4.5 times as great in the last 2 hr as between the 5th and 7th hr. With plum roots, cyanogenesis was only slightly greater in the last 2 hr than in the 5th to 7th hr.

HCN was not produced by roots of any of the 3 species at 27°C during 8 hr of exposure to air or to atmospheres composed of 10% O₂-90% N₂ or 5% O₂-95% N₂. Thus, conditions causing cyanogenic glycoside hydrolysis appear to exist when the O₂ level in the root environment is below 5%. HCN production was due to the conditions imposed, since that evolved after cutting either diminished greatly or stopped. Whether anaerobic conditions *per se* are necessary for glycoside breakdown was not determined.

Temperature affected the rate of cyanogenesis from detached roots in a manner similar to its effect on sensitivity to waterlogging (Fig. 1). Because of the low amounts of HCN released by plum roots at 22°C it is assumed that rates would be even lower at temperatures below 22°C. Thus, a threshold for HCN evolution in the absence of O₂ appeared to exist with plum at approximately 22°C; with peach and apricot it was below 17°C. With plum, this threshold coincided with a marked decrease in sensitivity to waterlogging between 22 and 17°C. Peach and apricot were still highly sensitive at 17°C. The lack of difference in cyanogenesis between peach and apricot, and the higher rates from both these species than from plum is consistent with a similar relationship among them in sensitivity to waterlogging (Fig. 1). The greater loss of cyanogenic glycoside from peach than from plum during waterlogging is also in line with the differences in HCN production by detached root systems.

From the results it appears clear that cyanogenic glycoside hydrolysis can be caused by anaerobic conditions during waterlogging. The inhibitory effects of HCN could lead to plant death. Thus, the greater tolerance of plum might be explained on the basis of lower amounts of hydrolysis. A more complex phenomenon than HCN inhibition alone, however, is indicated by the lack of relationship between glycoside content and both hydrolysis during waterlogging and cyanogenesis in N₂. Where peach roots contained 1.4 times as much glycoside as plum, the rates of HCN evolution in N₂ were approximately 10- to 7-fold as great at 22 and 27°C, respectively. Thus, it appears that peach, and probably apricot, are also more sensitive to conditions causing cyanogenesis and that processes leading to hydrolysis are stimulated more readily in these 2 species than in plum. An autocatalytic process is suggested by the exponential relationship between cyanogenesis and temperature, and also time, in both the 8- and 25-hr experiments. It is proposed, then, that glycoside hydrolysis and resultant HCN inhibition were secondary to other effects of O₂ deficiency. Also, cyanogenesis could not account for differential sensitivity among species without cyanogenic glycosides.

Although there are numerous ways in which the lack of O₂ could adversely affect roots, such damage is thought to be secondary to effects on respiration and energy-producing mechanisms (1, 2, 9, 16). β-glycosidases and their substrates, in this case cyanogenic glycoside, are separated spatially in normal cells and tissues (17, 18). With peach, the glycoside is located in the outer root tissue (19). For hydrolysis to occur, either physical damage or autolytic disorganization is required (10, 16). The release of HCN upon physical damage to organs containing the parent glycoside is well known, and occurred here

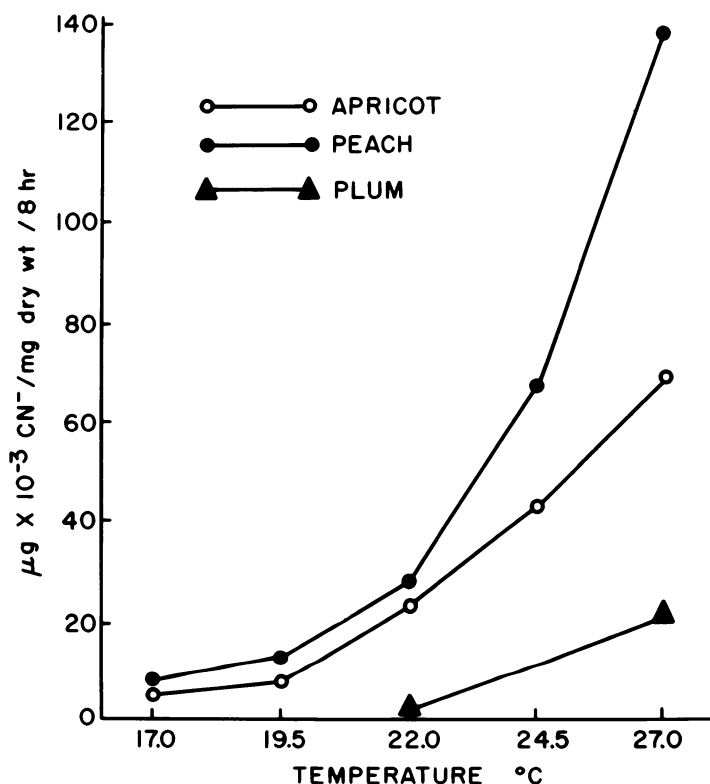


Fig. 2. The effect of temperature on cyanogenesis by detached root systems of peach, apricot, and plum seedlings during 8 hr in N₂.

when root systems were cut from the plant.

The maintenance of cellular membranes is considered to be dependent upon energy from aerobic respiration (1, 9, 16). Root cells have been shown to release their contents to their environment during brief anaerobiosis (7). Anaerobic conditions would be expected to cause membrane disorganization by reducing the source of energy from respiration. Retention of selectively permeable membranes may be considered essential for separation of cyanogenic glycosides and their hydrolytic enzymes. It is therefore suggested that, under anaerobic conditions during waterlogging, respiration and resultant energy transfer are inhibited initially. With a deficient supply of energy for maintenance of membranes, cellular disorganization occurs. As a result, the cyanogenic glycoside and its hydrolytic enzymes come in contact and hydrolysis takes place. The HCN so released may cause additional inhibition and cellular damage, thus increasing severity of plant response in an autocatalytic manner. The lower sensitivity of plum than of peach and apricot might be explained by differences in respiratory mechanisms, with secondary effects due to cyanogenesis. The characteristics of plum responsible for its overall greater tolerance to waterlogging are probably of a quantitative rather than qualitative nature. This is indicated by the variability in sensitivity among individual seedlings (Fig. 1) and the high amount of glycoside hydrolysis in the plant that died during treatment in the controlled-environment room.

Cyanogenesis appears to be a highly sensitive indicator of cellular damage and relative sensitivity to waterlogging. Measurement of HCN evolution under limiting availability of O₂, and as influenced by temperature, should be useful in selecting rootstocks of species containing cyanogenic glycosides which might provide increased tolerance to waterlogging.

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Analysis of Low Temperature Stimulation of Floral Initiation in Poinsettia cv. Paul Mikkelsen¹

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Abstract. The floral initiation response of 'Paul Mikkelsen' poinsettia to low temperature under long days was saturated after exposure to constant temperatures of 60°F for 10 days. Low temperature was perceived by the shoots but not the roots. As has been reported by others, high temperatures (80°F) during and after short days inhibited floral initiation. Gibberellin A₃ was an effective inhibitor of low temperature stimulated floral initiation under long days and Cycocel promoted flowering under long days at 70°F but not at 80°. Neither light source nor intensity greatly influenced low temperature stimulated floral initiation. These findings are discussed in relation to a possible mechanism by which low temperatures stimulate floral initiation in this short day plant and in relation to cultural practices.

Poinsettia (*Euphorbia pulcherrima* Willd.) is a short day plant in which the critical daylength for floral initiation can be altered by temperature (11). The cv. Paul Mikkelsen is particularly sensitive to temperature and when grown at 60°F night temperature will initiate floral primordia regardless of photoperiod treatment (10). A similar promotive effect of low

temperature on flowering of short day plants under long day conditions has been observed in *Pharbitis* (9) and *Fragaria* (2). In both species floral initiation in long days is promoted by treatment with the growth retardant Cycocel (3,16) as well as by low temperature. Thompson and Guttridge (14) have reported that floral initiation in *Fragaria* is inhibited by applications of gibberellin A₃ (GA₃). A GA₃ induced delay of floral initiation in poinsettia under short days has been reported by Guttridge (4).

In contrast to the photoperiodic control of flowering, alternate environmental controls of floral initiation (such as low

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