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A contribution to the knowledge of drought resistance of Mediterranean pine trees[1]

by

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A. Introduction

The genus *Pinus* is known as very resistant to adverse climatic and soil conditions. *P. silvestris* grows on the Kola peninsula near 70° of northern latitude. Also *P. cembra* in Siberia and the Alps resists extreme cold and water stress connected with it, in the winter time. The shrubby *P. mughus* is a pioneer above the timber line.

Hardly less baffling is the resistance of pines to summer drought in the Mediterranean basin and the Near East. In earlier articles, the senior author has shown that in late summer, Aleppo pine needles cut down their transpiration to extremely low levels (Oppenheimer, 1932; 1947).

Recently we have carried out experiments with different Mediterranean pines, in order to collect data on the drought resistance of their needles. Isolated needle bundles were exposed to desiccation under controlled conditions and their change in weight was recorded during several days to one week. Being interested in "lethal" drought resistance in the sense of Stocker (1956), we endeavoured to find out how much water detached needles can lose before a "permanent damage point" or "Schadenspunkt" (Huber and Ziegler, 1962) is reached and, consequently, how long they can keep on without water supply, i. e. what their endurance ("Ausdauer"; Pisek and Berger, 1938; Larcher, 1957) is at the transpiration intensity of the experiment. Another point arousing interest was the role played by stomatal regulation of water expense.

Of the two species compared in the present report, *P. pinea* has a mainly South European distribution area. The indigenous character of its stands in the Lebanon is controversial, but Feinbrun (1959) accepts it. In Israel and Jordan, it is certainly introduced; it succeeds best in the coastal region (Acre, Mt. Carmel, Jaffa). The other, also omni-Mediterranean species, *P. halepensis*, is known as much more drought-resistant than the former. It grows wild even in North-Africa and in Palestine where it can be cultivated even in semi-desertic surroundings.

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[1] Study carried out under Project No. A 10-FS 7, U. S. Forest Service, Department of Agriculture, Washington, D. C., whose help is gratefully acknowledged.

Since in conifers, like pines and cypresses, the capacity to restrict transpiration certainly plays an essential part in their total or "constitutional" drought resistance (STOCKER, 1956), the following analysis of drought avoidance and tolerance of pine needles should prove interesting. Though we by no means agree that, generally speaking, drought resistant spermatophytes must be expected to possess more drought tolerant leaves than nonresistant ones, this nevertheless might be a generic property and apply sometimes to nearly related species of the same genus.

B. Material and methods

The brachyblasts used were detached from trees of the arboretum of the Faculty of Agriculture. The latter had been planted in 1941. The brachyblasts called here also needle bundles were well developed and healthy though the rains in November and December, 1962 were scarce, weather mainly hot and dry, and the soil far from saturated. The brachyblasts derived all from the preceding spring's flush.

Before the experiment, they were saturated by putting them firmly on water-saturated cotton in closed petri dishes where they remained for 24 hours. After sealing the open basis by a blob of vaseline, they were stuck into plastic nets kept in an automatically controlled "Vapour-temp" humidity chamber kept at 27°C and 84 ± 1 % relative humidity. The chamber is a ventilated cylindrical bell-glass about 45 cm across. It was illuminated during the day by the near-by windows of the room and at night by four fluorescent tubes from the ceiling. The temperature and humidity conditions resemble those prevailing on summer days on the shores of the Mediterranean Sea.

In the second elaborate trial of two upon which we rely mainly in this report, two composite samples of 20 brachyblasts each of either species were used for the study of transpiration which continued for one week. 13 samples of 20 brachyblasts each served for the determination of increasing water loss and decreasing resaturation capacity during times varying from one half to 170 hours. Further 13 times 7 bundles from the same flush of the same trees served for vitality and stomatal tests. These were carried out each time when the dehydration of one of the samples was interrupted for recording of weight or broken off, for resaturation.

Each needle bundle was weighed separately on a torsion balance, its weight being recorded reiterately in the transpiration tests, but at the beginning and the end of the progressively longer dehydration periods only in the resaturation tests.

After exposure to the unsaturated atmosphere of the chamber, the brachyblasts to be tested for resaturation capacity, were put again on moist cotton in petri dishes. As the senior author points out elsewhere (OPPENHEIMER, 1963), leaves of evergreen trees and shrubs regain approximately their original weight during about 12 to 24 hours in a moist chamber if they have not been damaged by previous dehydration. If, however, their water saturation deficit (STOCKER, 1929) surpasses a critical value which we propose to call "permanent turgor loss point" (PTLP), their rehydration weight is lower than their saturation weight was at the beginning of the experiment provided that no infiltration of the intercellular spaces with water has taken place. The difference between both values was defined as "water resaturation deficit" or WRD and is calculated by the formula

$$\mathrm{WRD} = \frac{W_{\mathrm{max}} - W_{\mathrm{resat}}}{W_{\mathrm{max}}} \cdot 100$$

where W_{max} is the presaturation water content of the unwilted, W_{resat} the water content of the rehydrated leaves or needles.

The final water saturation water deficit WSD of the "wilted" leaves or needles at the end of their time of exposure to dry air is calculated for each sample by aid of STOCKER's, slightly modified formula

$$\mathrm{WSD} = \frac{W_{\mathrm{max}} - W_{\mathrm{fin}}}{W_{\mathrm{max}}} \cdot 100,$$

where W_{fin}, equivalent to STOCKER's W_{nat}, the water content under natural conditions in ecological work, designates here the final water content attained during the trials, at the end of the dehydration period of each sample.

The water resaturation deficit is a reliable index of damage caused by dehydration. It rises the more, the more the permanent turgor loss point has been overstepped during the trial. Its applicability to pine needles has been established in earlier unpublished experiments.

As check methods for establishing drought damage, we used: (1) 1 % triphenyl tetrazolium chloride solutions where crosswise cut, 5 mm long segments from the middle of the needles, split lengthwise or not, are immersed in the dark for 24 hours (for details see PARKER, 1951). (2) Vital staining and plasmolysis of longitudinal, not too thin hand sections through the central portion of the needles. The sections were kept for 4 to 24 hours in 2-molar sucrose solution stained with neutral red (1 : 10,000).

In the first experiment (November 19th—31st 1962), we used the first, in the second which began on January, 29th 1963, the second check method. However in both, as well dehydrated needles as rehydrated ones (24 hours) were tested, in order to establish if damage indicated by the tests was reversible or not.

At the end of the trials, the brachyblasts were dried in an oven at 70°C for 24 hours and their dry weight was determined. Their water content at saturation was calculated and diagrams of transpiration were drawn.

For stomatal tests, the needles were dipped into a solution of Crystal Violet for a few minutes. 0.8 gram of this stain were dissolved in 2 ml of ethoxyethanol; to the solution 9 ml of chloroform and 9 ml of ether were added. After the bath in a test tube, the needles were washed in secondary butyl alcohol. Open stomata are marked by deep violet circular spots surrounding them and often coalescing along the longitudinal lines marking their natural arrangement. No infiltration is expressed by zero; few isolated spots by 1; numerous spots by 2; very numerous, often coalescing spots staining considerable areas, by 3; widespread or total coloration of the needles by 4. Intermediary figures result from differences between degrees of infiltration in different portions of one and the same needle or in different needles forming a composite sample of five. For details, the reader is referred to OPPENHEIMER and ENGELBERG (1962). Needles of *P. pinea*, in contrast to those of *P. halepensis*, rendered no quite satisfactory results. Large scattered areas stained deep violet, not the less, but the more the needles had been dehydrated, i. e. when no single infiltrated spots appeared indicating stomatal opening. We suppose that this was the consequence of cracks and fissures formed in the epidermis by contraction during drying or possibly of not careful enough handling with the forceps. We disregarded these areas in the evaluation of the results.

In the calculation of water deficits, we assumed that dry weight changed but little during the experiment and that consequently figures established at its end could be used also for the calculation of the initial water content. This seems justified since the dry weight diminished mainly during the preliminary saturation of the needles, i. e. before the beginning of the trials. Later on, in the humidity chamber, a sample of *P. pinea* lost in 77 hours only 0.6 % of its fresh weight by respiration. Its dry weight dropped during these hours from 32.7 to 32.1 % on a fresh weight basis. Corresponding loss in *P. halepensis* was 1.3 %, the difference between 41.2 and 39.9 %. Resulting errors in the determination of maximum water content will not reach 2 % in the first and be smaller than 2.5 % in the second case. Further calculation showed that in turn, this will produce errors of WSD's and WRD's within the limits of 1 %, as long as either does not surpass 45 % of initial water content, i. e. as long as the WSD remains within the range of figures decisive for the determination of the PTLP.

C. Results

(a) Transpiration

Diagrams 1 and 2 show the course of transpiration in the second experiment, expressed, respectively, as hourly or total percentage of the initial water content. Transpiration sets in with high figures of 12.3 % during the first hour in *P. halepensis* and 11 % in *P. pinea*. A steep fall follows reducing figures to 2—4 % in the next three hours. In *P. pinea* needles, transpiration drops more drastically, arriving at a level of 2—3 % already after 1½ hours,

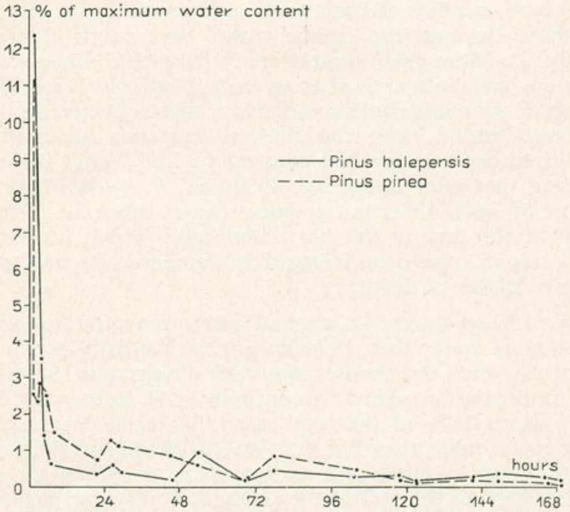

Fig. 1. Hourly transpiration, expressed as percentage of maximum water content of excised brachyblasts of *Pinus pinea* (— — —) and *Pinus halepensis* (———) in the course of one week.

while *P. halepensis* still spends 8.4 % at the same time. Thus needles of the latter have lost an average of 20 % of their initial water content already at the end of the third hour, those of *P. pinea* only 10 %. While this difference is typical, individual variations are very considerable.

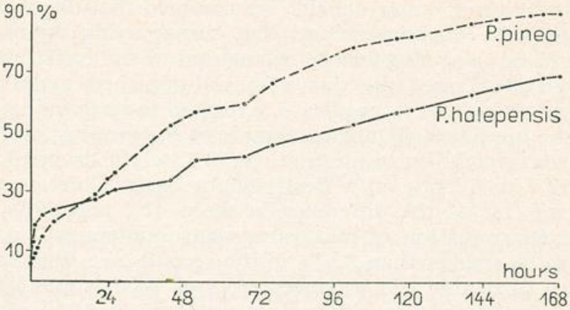

Fig. 2. Accumulative (total) transpiration of the same brachyblasts.

The drop continues in the next four hours when, however, the greater capacity of restriction of *P. halepensis* changes the picture completely. After 7 hours, the latter has arrived at a level of only 0.5 % per hour while *P. pinea* needles still spend 1.6 % per hour. Stomatal tests after 8 hours indicated that by now stomata had closed completely in *P. halepensis*, while in *P. pinea* a few stomata were still open here and there (mark 0.2). This stood in marked contrast to the condition at the beginning of the experiment when numerous stomata (mark 2) had been found open in both species, and during the second and third hour, when a tendency towards wider opening-corresponding to the relatively higher transpiration-had, on the contrary, become apparent in *P. halepensis*.

After 27 hours, needles of both species which now, for all practical purposes, lose water through the cuticle alone, have spent about $^{1}/_{3}$ of their total water (fig. 2). More exact figures are 30 % with *P. halepensis*, 34 % with *pinea*. They now have both arrived at an early dangerous level of dehydration. However, since *P. halepensis* needles are much more effective in the restriction of cuticular transpiration, their transpiration fluctuates during the second day about 0.4 % in an hour, in marked contrast to 1.0 % in *P. pinea*. As a consequence, we find that after altogether 45 hours, *P. halepensis* has spent only a total average of 34 % of its initial water but *P. pinea* 52. This means that the condition of the former has not deteriorated much further while most of the latter's tissues have been irreparably damaged. Its needles are already in a progressive phase of death.

Beginning with the third day, we find a low transpiration in both species. *P. pinea* loses more water than *P. halepensis* till the fifth day but less on the sixth and seventh when the needles approach air-dryness (fig. 2). In contrast, control over transpiration seems to continue in *P. halepensis* till the end of the third day when 44 % of the total water has been spent. In the further course of the experiment, they lose 0.2—0.4 % of their initial water per hour and — with 30 % of the latter still at their disposal — they are still far from complete desiccation at the end of the week. According to earlier experience, the needles might have been kept for more two or three weeks before losing completely their natural bright green colour and flexibility.

It seems interesting that average transpiration of the needles of both species fluctuated considerably with time from the end of the first to the fourth day. Since this apparently cannot have been produced by stomatal movements, it might have been the consequence of recurring waves of cell death in tissues differing in their tolerance to desiccation. As is well known, dead tissues spend water at a higher rate than living ones, and often death is marked by a rise of transpiration.

(b) Drought tolerance and avoidance

One of the most problematic items in the theory of drought resistance of conifers is the question when actually death of the gradually drying needles occurs. No clear-cut and generally recognized symptoms demonstrate this. It is PARKER's (1951) merit to have introduced the tetrazolium test for the decision of this problem. However, the formation of red formazan indicating the living state declines gradually; the complete absence of the red colour after the test indicates therefore a final phase of a prolonged agony setting in much earlier.

A much more adequate answer is given by our resaturation test which rendered quite similar results in both experiments. Fig. 3 and 4 show that needles of both species react in most cases to a maximum loss of 25 % of their initial water by a resaturation deficit of less than about 10 % indicating no or very slight damage. With further rise of the final saturation deficit the resaturation deficit becomes progressively greater indicating that the „permanent turgor loss point" — which is probably identical with HUBER and ZIEGLER's (1962) "Schädigungswert" — has been surpassed.

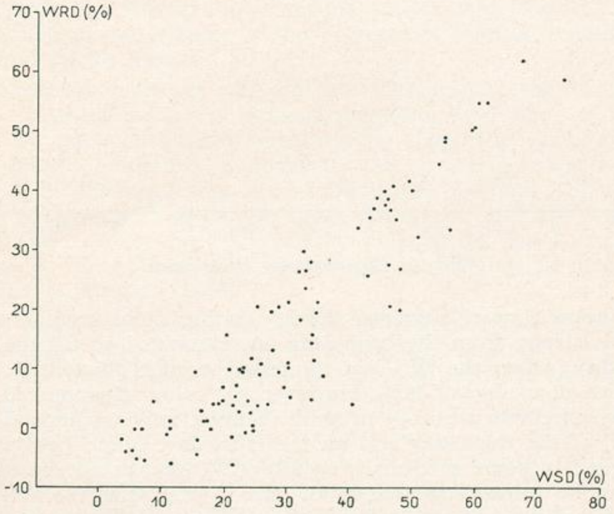

Fig. 3. Water Resaturation Deficits of *Pinus pinea* needles (ordinates) in dependence upon the earlier final Water Saturation Deficit reached after dehydration (abscissae). The figures have been pooled, irrespective of time of dehydration.

This conclusion is corroborated by the tetrazolium tests for *P. pinea* but not for *P. halepensis*. Lengthwise cut needle segments of the former stained red throughout in all tests in the first 14 hours of the second experiment when the WSD did not pass beyond an average figure of 25.3 %. But from the 19th hour, with an average WSD of 23.3 %, no red colour appeared except at and around the central vascular bundle. Formazan formation-never observed in the interior of non-split segments, but only near its ends, — also diminished very much in this phase of dehydration. After 48 hours, with WSD reaching an average of 31.6 %, staining of the non-split sections became restricted to the cut surfaces alone, not spreading inwards at all. In all later tests, after three to seven days of exposure to unsaturated air, and with average figures of WSD above 40 %, no or only a very faint coloration was recorded.

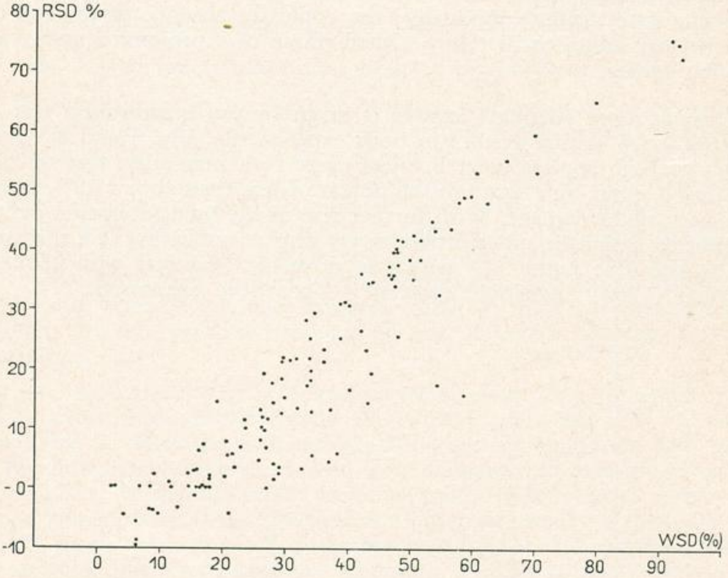

Fig. 4. The same for *P. halepensis*.

P. halepensis showed a greater vitality during these tests. Formazan formation was strong from the beginning and remained so till the end of the second day, when the WSD of the tested needles fluctuated between 30 and 70, with an average of 48 %. However, the colour disappeared completely or became extremely weak — in spite of practically unchanged WSD's — at the end of the third day and on the fifth day. Some faint staining was still observed in some sections even after 5¾ day, at an average WSD of 45.4 %, but no colour appeared at all after a week with the WSD rising to 73 %. These results seem to indicate that (1) no serious damage is produced during the first two days, i. e. as long as one half of the initial water content remains, but (2) this and higher losses prove fatal in the further course of the experiment.

Such conclusions stand, however in marked contrast to the results of the just mentioned resaturation tests which indicate that needles of both species do not differ appreciably in their drought tolerance and that irreparable turgor loss begins in the needles of both at 25 % WSD or even a little earlier. That the latter method is very reliable, is borne out by the microscopic examinations carried out during the first experiment, on November, 19th to 31st. They demonstrated clearly that internal damage sets in also in needles of *P. halepensis* very early though further water loss affecting the peripheric tissues begins later than in *P. pinea* and, consequently the ultimate destruction of these organs requires more time.

Our observations warrant the statement that the endodermis of the needles is the first tissue affected. The protoplasm of its colourless cells is densely packed with equal-sized tiny drops of an oil-like liquid, possibly an oleoresin which in healthy cells vibrate in Brownian molecular movement. When the water loss reaches about 15 % in both species, the movement stops and the cell content becomes opaque and brownish. The next tissue affected is the transfusion tissue. In contrast to the opinion of even modern authors, a large proportion of its cells is living and can easily be plasmolysed and vitally stained in longitudinal sections. In particular, this is easily achieved in the larger cells which are contiguous to the endodermis.

In both species, we observed a marked reduction in the number of plasmolysed and vitally stained cells when the WSD reached about 25 %. This occurred in the tested needles of both species after 24 hours. The endodermis was certainly dead at this stage.

In the further course of our observations which continued for five days, with average WSD's between 20 and 50 %, we always found slighter or heavier damage in the transfusion tissue. After 48 hours, we saw in *P. pinea* only a faint pink coloration in a few, non-plasmolysed cells, but in *P. halepensis* already complete disintegration of the protoplasts though in either species the WSD had hardly reached 30 %.

While these observations demonstrate clearly that the water storing transfusion tissue suffers heavy damage increasing with time at the PTLP established by our concomitant resaturation tests, the peripheric tissues comprising mainly epidermis, hypodermis and apparently also the adjacent radial rows of the chlorenchyma, were found more resistant. Apparently they cover their water deficits from the inner tissues in an early phase of dehydration while later on the chlorenchyma cells shrink and collapse, as can be seen already under a binocular stereoscope magnifying 30 times and was already stated by PARKER (1952).

Also the innermost bundle tissue was often found very resistant. Thus xylem parenchyma cells of *P. pinea* stored some neutral red even after five days when macroscopic tetrazolium tests rendered negative results.

In a final stage of desiccation, life expires also in the epi- and hypodermis, as is demonstrated by the absence of vital staining. It is difficult to arrive at a clear judgement on the condition of the green tissue. It rarely shows general plasmolysis and vital staining even in fresh needles while the decomposition of chlorophyll indicating death is observed only in a progressive phase of dehydration, if at all. In longitudinal sections of heavily droughted needles, we often observed that one side had become yellowish brown while the green colour persisted on the opposite flank.

So far, we have spoken only of needles investigated for vitality immediately upon their exposure to drought. But, as mentioned before, also needles which had been put back into moist conditions after such exposure, have been tested. The result often was a marked improvement of their vitality which could be demonstrated by both tetrazolium and neutral red-plasmolysis tests. Thus in the second experiment, needle segments of *P. pinea* resaturated after 48 hours of exposure in the drought chamber, developed a stronger colour in a larger area when tested with TTC (triphenyl tetrazolium chloride) than non-saturated segments. The difference remained quite consistent during the following days, i. e. resaturated segments evinced some faint reddish coloration while most non-resaturated did not show it. Likewise, split segments of resaturated *P. halepensis* droughted for four days which had reached a final WSD of 47 %, stained strongly over the whole cut surface, suggesting a return to life even of the transfusion tissue while comparable non-resaturated segments stained weakly only at the central bundle.

Some improvement of the condition of surviving cells by rehydration was also observed in sections of *P. pinea* immersed in sucrose and neutral red. Thus the number of vitally stained cells was much greater in sections of a needle droughted for 24 hours (calculated WSD 23.4 %) after rehydration, than in a non-rehydrated one. *P. halepensis*, however, showed no improvement at all in these microscopic tests.

D. Discussion and Conclusions

The present investigation corroborates earlier results (OPPENHEIMER 1932) concerning the rather "oligohydric"2) transpiration level — termed earlier "träge" by STOCKER — of the Aleppo pine. Maximum figures reaching only 140 mg per gram of fresh weight in an hour found earlier are matched now by initial intensities at saturation of 12 % of the maximum water content per hour (and by only 6 % in the first experiment). If we assume a 60 % water content per fresh weight at saturation, the latter figures are equivalent to 36—72 mg per gram and hour, on the fresh weight basis.

Under conditions of an adverse water balance, transpiration is reduced very much, though in an early stage of water stress, the needles "take the risk" of an appreciable water deficit, before stomatal restriction sets in. This is a typical behaviour of plants adapted to sunny habitats in general and to light demanding forest trees in particular. Thus the branches of the Norway spruce (*Picea abies*) studied by PISEK and TRANQUILLINI (1951) behave so if adapted to high light intensities. By comparison *P. pinea* restricts transpiration much earlier. Its needles seem less appropriate to withstand prolonged desiccation.

After 68 hours transpiration of *P. halepensis* reaches the very low level of about two of thousand of the initial water in an hour equivalent to 1.2 mg per gram and hour of fresh weight. This figure is even lower than cuticular transpiration of *P. silvestris* quoted by PISEK and BERGER (1938) who found 1.53 mg/g. h. In so far unpublished studies we found still three times lower figures in the Aleppo pine, while LARCHER (1957), studying branches of *P. cembra* in Austria, under winter conditions, quotes a daily loss of about 5 mg per gram and day only, for last season's needles spelling 0.2 mg/g. h.

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2) The senior author proposed this term in 1953.

These findings underline the senior author's earlier statement (1960, p. 130) that the stress imposed on the water balance of conifers of the cold zone during the winter time might be heavier than that suffered by Mediterranean conifers during the dry summer.

The resemblance of our results regarding the changes of cuticular transpiration with time and, more definitely, during the first 3 days with those of PISEK and BERGER (1938), is also far reaching. These authors found unexplained and very pronounced fluctuations under controlled conditions in *P. silvestris*. We find such "jerks" in both species investigated at the end of the first and a few hours after the third day, in *P. halepensis* also at the end of the second though our weighings were too infrequent to say exactly when they occurred and how long they lasted.

As mentioned above, it is quite possible that such rises above the average level — if not produced by submicroscopic stomatal movements — are brought about by successive waves of cell death in progressively more drought tolerant tissues. PRINGSHEIM (1931) has pointed out that "incipient drying" of cell walls in wilting leaves restricting transpiration is replaced at the moment of cell death by free imbibition. The dying protoplasts release solutes and water alike, and transpiration rises. If, as he explains, the cell walls are thick, no steady flow through their narrow and long intermicellary channels can be expected and a fluctuating course of transpiration must result. His reasoning applies also to needle leaves with their thick-walled external tissues absorbing water from dying cells of the core.

In addition to the effect of cutinization, the external tissues might be protected from early desiccation also by the rigidity of their very thick and lignified cell walls. This would be expected to produce cohesive tension in the water of both protoplasm and vacuoles upon water loss and to reduce vapour pressure at the cell's surface to low figures (PRINGSHEIM, 1931).

Regarding the level of critical water loss, we refrain from discussing here the quantitative resaturation method which was developed by the senior author during his studies on Mediterranean broad-leafed evergreens (OPPENHEIMER, 1963). The point diagrams of fig. 3 and 4 have been drawn irrespective of the time factor, but it must be stressed that the same WSD was reached in our trials after widely different times of droughting. It is quite possible that the correlation of WSD with WRD would be still more pronounced if the time of exposure to drought had not been disregarded, a shorter exposure producing, in general, a smaller WRD at the same level of dehydration. On the advice of Dr. PUTTER, statistican of the National and University Institute of Agriculture, Rehovot, we refrained from calculating a linear regression line, beginning with the PTLP, as we did in the evaluation of our results with other sclerophylls where such a relationship was more acceptable. Incidentally, we have found the method is applicable to Cupressoids, as *Cupressus* and *Biota*.

On the other hand the level of initial damage established in the present investigation, requires comment. It is interesting to compare our results with those of MARSHALL (1931) who studied drought resistance in seedlings of Scotch pine. This author established damage when 13 to 30 % of the initial water content had been spent. The figures rose with hardening and age. Corresponding values for *P. ponderosa* were 21, for *P. echinata* 22, comparing favourably with the results of our own resaturation and vital staining tests. MARSHALL who relied on change of colour to a brighter green and on wrinkles appearing on hypocotyl and cotyledons, found higher drought tolerance in *P. monticola* and *P. caribaea* seedlings. Critical figures of WSD were 36 and 49 %, respectively. His method was, however, too primitive to render very accurate results.

PARKER's work on the needles of *P. strobus* and *P. nigra* (1952) is better comparable to our studies. The author observed early damage in needles kept for several weeks in drought chambers when 10 to 15 % of the fresh weight had been lost. This range corresponding approximately to 15—23 % of the initial water, is identical with the upper range of reversible water loss in our resaturation tests. We suppose that the endodermis was heavily, the transfusion tissue slightly damaged in PARKER's experiments though he did not notice it. The TTC-test became negative in both species when 25 % of the fresh weight — equivalent to about 40 % of initial water — had been lost by transpiration. This again agrees well with our own results with *P. pinea* and even *P. halepensis* though in the latter species, formation of formazan became apparent in several needles even after 52 hours, at WSD's about 48 %. The agreement became still better after 6 days when practically no formazan was observed in our *P. halepensis* needle segments at WSD's of about 45 %.

The comparison suggests that the first diminution of staining intensity in the tetrazolium test coincides in different species of pines more or less with the permanent turgor loss point of our terminology, while the first WSD where no reduction of the TTC takes place any more, marks a "lethal point" for the majority of the tissues composing the needles but not for the few, very resistant ones, as the xylem parenchyma which still might stain red if split needles are used.

On the other hand, there is no agreement between our results and those of the Innsbruck workers determining the "sublethal" water deficit of pine and other conifer needles. This concept proposed earlier by one of us (OPPENHEIMER, 1932) can no longer be maintained as a measure of true drought resistance, as we point out in another article (OPPENHEIMER, 1963). The appearance of necrotic spots on leaves used earlier as index of early drought damage, is neither a trustworthy indication that serious damage did not set in before, nor do such spots appear in all leaves. Putting back to water, practiced by PISEK and BERGER (1938) gives a correct answer on vitality only if the permanent turgor loss is concomitantly followed up by weighings. The time has come to be very careful in the interpretation of such tests, and it seems justified to rely mainly on cell physiological work, as SULLIVAN and LEVITT (1959) did. In particular, our results disprove the trustfulness of simple criteria, as minor changes of colour, breaking of the needles upon bending, turpentine odour which, as PARKER (1951) maintains, coincide with the "lethal point". To give an example, needles of *P. halepensis* remain quite flexible even when approaching air-dryness. The "lethal point" is a fiction since death of leaves is a prolonged process with initial, progressive and final phases.

Finally let us consider what can be concluded from our experiments regarding the relative drought resistance of the investigated species. Our study shows that detached needles of the Stone Pine (*P. pinea*) suffered serious damage already in the first 24 hours of the trials. The same applies to the Aleppo pine but the needles of the latter die much more slowly, thanks to their greater capacity to restrict transpiration, i. e. their "drought avoidance" in the sense of LEVITT (1956), even in a damaged condition. It seems premature to undertake an evaluation of the ecological implications of this specific difference. Obviously the greater drought avoidance of the Aleppo pine needles will prove helpful only if early damage in the endodermis and the transfusion tissue is either reversible in needles attached to the tree, in contrast to excised ones. Or, if alternatively, the needles possibly remain functional and are not prematurely shed even if such early damage persists.

Ample data collected by BERLINGER (1956) on Mt. Carmel clearly demonstrate that, at least in this region, natural WSD's of Aleppo pine needles never rise beyond 22.6 % throughout the summer. In analogy, our own (1932) late summer figures collected on Mt. Scopus (Jerusalem) on branchlets and brachyblasts rose beyond 24.2 % only in one isolated brachyblast from a heavily wilted tree. Even if the latter figures might have been a little too low, for methodological reasons, they strengthen the impression that healthy needles of *P. halepensis* rarely lose more than 25 % of their saturation water, in the natural area of distribution of the species. It seems quite possible, therefore that higher losses spell damage even in the forest. If this is true, shedding of old needles which have lost more of their normal water content than the younger ones, may be a widespread consequence of prolonged dry spells, adjusting the trees' water balance to the reduced water supply from the soil.

The similarity of the permanent turgor loss point in the pine species investigated so far by us including *P. radiata*, suggests that it is genetically fixed in a rather narrow range. Conversely the capacity to restrict water loss varies very considerably. We find that needles of *P. halepensis* are more drought avoidant than those of *P. pinea*, the latter more than *P. brutia* while needles of *P. radiata* can hardly be called drought avoidant drying up quite easily, in spite of early closure of their stomata (OPPENHEIMER and ENGELBERG, 1962). The needles of these species differ considerably in their texture. It is quite possible that drought avoidance produced by structural properties, like more or less developed intercellular spaces, more or less heavy thickening and cutinization of the epi- and hypodermal layersare correlated with resistance to summer or frost drought if not with both.

The study of drought avoidance and the correlated structural properties of the needles might also prove useful for the understanding of ecological adaptation and geographical distribution areas (PARKER, 1951). Thus the highly drought avoidant *P. halepensis* distinguished by a heavily cutinized epidermis and a very densely arranged chlorenchyma tissue, penetrates into semi-arid areas, while the little drought avoidant *P. radiata*, a tertiary relic, with less heavily cutinized needles and rather large intercellular spaces in the green tissue, clings to the Oceanside of California and is known to foresters as unable to grow in arid zones with a low rainfall.

In PARKER's (1952) experiments with *P. strobus* and *P. nigra* the former was found to possess the more drought tolerant needles, in spite of the more humid character of its area of distribution. However, this is no valid argument against the above hypothesis. In the Mediterranean basin, *P. nigra* is much less drought-resistant than the Mediterranean species studied by us while *P. strobus* is exposed to heavy frost drought in its native area.

We are, of course, fully cognizant of the fact that drought avoidance of leaves is only one component of total drought resistance. Its usefulness seems further to be restricted in pine needles by the rather low PTLP. There might exist also other mechanisms restricting water loss, still to be explored. Nevertheless we feel that needle structure (GRAHLE, 1953) and needles' drought resistance, in connection with climate and geographical distribution, are worth of further attention.

Summary

The points of damage and death of conifer needles by drought are not exactly known, for lack of adequate methods for their determination. To further the solution of this long debated problem, needles of two pine species somewhat differing in drought resistance, were exposed to dehydration tests. Transpiration of previously saturated needles of *Pinus pinea* and *P. halepensis* dropped during the first hours to figures about ten times lower than the initial ones, by stomatal regulation. This drop set in earlier in the needles of *P. pinea*. From the fifth hour to the end of the fifth day, cuticular transpiration of *P. pinea* was considerably higher than that of *P. halepensis*, the needles of which, consequently, dried up much later.

Vitality of the needles during dehydration was investigated by resaturation capacity, macroscopical tetrazolium tests and microscopical observation for plasmolysis and vital staining. Early damage appeared in both species at a "permanent turgor loss point" (PTLP) when about 25 % of the saturation water had evaporated. This was accompanied by serious damage in the endodermis and the transfusion tissue. When dehydration deficits rise to higher levels, the capacity of the needles to imbibe water is more and more impaired. This produces "rehydration deficits" that rise gradually, as a consequence of damage and death of an increasing number of cells, even in rather drought tolerant tissues. Cells of the central vascular bundle and of the peripheric protecting tissues proved relatively resistant.

Triphenyl tetrazolium chloride tests rendered similar results as the above methods in *P. pinea*, but indicated a higher degree of vitality in damaged needles of *P. halepensis*. Drought avoidance seems to play an important part in the total drought resistance of the investigated species although their drought tolerance apparently is low and quite similar in both.

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