INVESTIGATIONS INTO THE SIGNIFICANCE OF PHOTOSYNTHESIS IN RESISTANCE TO PLANT DISEASES.

Onni Pohjakallio, Laila Ollila and Kerttu Paasi

Institute of Plant Pathology, University of Helsinki.

Received 1th June, 1951.

The photosynthesis of a plant has been found to increase the spore formation of a rust fungus parasitizing on it (2, 3, 7, 8, 9, 14, 15). Lack of light lengthens the incubation period of rust fungi and weakens spore formation (1, 3, 4, 5, 8). However, rust fungi grow and form spores in darkness also, provided sufficient carbohydrates are available (4, 7, 8, 12).

Gäumann (6) assumes, however, that the tendency of the lack of light to increase the rust resistance of plants is due in the first place to the weakening of the host plant, which results in hyperergic reactions leading to necrotic resistance. On the other hand, Sempio (12) reports cases where abundant light at the initial stages of infection strengthens the defensive reactions of plants against certain rust fungi and powdery mildews. The intensification of defence has expressed itself in the strengthning of photosynthesis and glycolysis of an infected plant as compared with respiration. These investigations evidenced that in continuous light the metabolic defensive reactions of wheat against *Oidium monilioides* are connected with an attack of the nucleus into the haustorium of the parasite, resulting in the degeneration of the haustorium. Sempio in fact assumes that the intensified spreading of *Oidium* disease due to dark nights results in the first place from the defensive reactions connected with photosynthesis discountinuing by night and nuclear reactions making themselves felt in conditions of long day only. On the other hand, Rodenhiser and Taylor (11) have shown that the lengthening of daylight weakens the resistance of wheat to *Tilletia levis* and *T. tritici* smut fungi. The wavelength of light has also been found to be of importance for the resistance of plants to diseases (cf. 6). Gäumann (6) reports, on the basis of literature, several examples of the modifying effect light conditions exert on the resistance of plants to diseases.

Investigations previously (10) carried out in the Institute of Plant Pathology, University of Helsinki, have shown that strong light increased the resistance of plants to *Sclerotinia trifoliorum*. The following is a report on certain additional investigations confirming the conception that there are phenomena connected with photosynthesis that may protect plants against attacks by parasites.

Botrytis cinerea PERS. in red clover.

When growing red clover (*Trifolium pratense* L.) in the laboratory, on pots covered by a glass bowl, grey mould fungus (*Botrytis cinerea* PERS.) appeared on the clover. *Botrytis* destroyed the clover growing in the shaded part of the pot, while the plants on the window side of the pots remained healthy. *Botrytis cinerea* was isolated on nutrient substratum, and the cultures obtained were used to infect (Oct. 9, 1950) small red clover seedlings (9 days after sowing) that had been grown in sterile conditions in test tubes sealed with cotton wool plugs, with quartz sand and KNOPP's solution as the nutrient substratum. A number of the test tubes were kept in darkness from the time sowing of the clover, a number were darkened 4 days before infection, and the balance were kept in light throughout on the laboratory window sill; the test tube cultures in each lot numbered 16, and half of them were infected. — The uninfected plants remained healthy throughout the test period (31 days), whereas all the infected plants became diseased and finally died. However, the plants died more slowly in light than in darkness (Table 1).

In the late winter of 1951 investigations were carried out into the direct effect of light on the growth and spore formation of *Bortytis cinerea*. The fungus was grown on Petri dishes, on nutrient agar substratum. The dishes were placed on a table, 2.5 metres from the laboratory window a) unshaded, b) shaded on all sides, and c) completely covered by a cardboard frame, 5 dishes in each group. Light intensity in cloudy weather, at noon, was: a) 3200, b) 150 and c) O lux. At the beginning of the experimental period the weather was cloudy; on March 4 and 5 and for a few days later the sun shone, about noon, for some two hours on the unshaded dishes, producing a light intensity of some 30 000 lux. As a result of this the temperature, which had been approx. +19°C for all experimental dishes in cloudy weather, rose on the exterior surface of the dishes to a) +25°C, b) +22°C and c) +21.7°C. Hence, plenty of water evaporated from the unshaded growth substrata, condensing in large

Table 1. Botrytis cinerea destruction in pure cultures of red clover (sown Sept. 30, 1950).

Date of infection	Darkened	Health of plants 0—10 (10=healthy; 0 = completely destroyed)					
		Oct. 9	Oct. 16	Oct. 20	Oct. 23	Oct. 25	Oct. 31
Oct. 9	Sept. 30	10	3	0	0	0	0
»	Oct. 5	10	5	1	0	0	0
»	nil	10	7	6	3	2	0
Not infected	Sept. 30	10	10	10	10	10	10
»	Oct. 5	10	10	10	10	10	10
»	nil	10	10	10	10	10	10

Table 2. Influence of light on the growth and formation of conidia of Botrytis cinerea mycelium (experiment started Feb. 28, 1951).

Date	Average diameter of mycelium, cm		
	Unshaded	Shaded from sides	Darkened
Feb. 28	0.00	0.00	0.00
Mar. 2	2.18	2.05	2.09
» 3	3.99	3.87	3.78
» 5	6.67	7.78	8.96
		Formation of conidia (0—10)	
Mar. 7	8	0	8
» 13	8	9	10
		Formation of sclerotia (0.—10)	
Mar. 13	8	10	7

Table 3. Resistance of green leaflet parts and those without chlorophyll, of red clover, to Botrytis cinerea (mean values of several tests).

Lapse of days from infection	Health of cells (0—10)		
	Green cells	Yellow cells	White cells
0	10	10	10
3	10	4	3
5	7	1	0

Table 4. Effect of light on the incidence of Pythium damping-off disease in radishes.

Infection conditions	Percentage of light intensity outdoors						Total of dead seedlings %
	67	53	46	38	33	30	
	Dead seedlings (%)						
On shooting							
with continuous additional light..	24,1	13,8	13,0	11,1	4,5	20,8	14,9
no additional light	12,1	16,0	8,8	20,7	21,2	13,8	15,3
After shooting							
with continuous additional light..	4,8	0,0	5,0	4,2	14,3	15,8	6,9
no additional light	24,1	9,5	19,4	43,5	53,8	48,0	32,9
Leaf infected							
with continuous additional light..	0,0	0,0	0,0	0,0	4,8	5,3	1,3
no additional light	3,4	9,5	3,2	0,0	19,3	24,0	8,2

drops on the lid of the dish. Only a few drops condensed on the lids of the dishes shaded on all sides, and practically none in the darkened dishes. Hence, towards the end of the experimental period, apart from light conditions, the results were also affected by temperature and humidity conditions.

On cloudy days, the growth of *Botrytis* mycelium was practically equally intense in all experimental dishes, which appears to indicate that diffuse light had no direct influence on its growth (Table 2). Sunny days (March 4—5) distinctly retarded the growth of *Botrytis* mycelium in unshaded Petri dishes, but it cannot be concluded with certainty whether this was due to light or the plentiful evaporation of water from the growth substratum. The formation of conidia also, in unshaded dishes remained slightly less than in the others. The shading was not found to have any consistent influence on the formation of sclerotia.

The influence of photosynthesis on the resistance of red clover to diseases was also studied by transferring mycelium and conidia of *Botrytis cinerea* to the leaflets of red clover which, either in part or in their entirety, had no chlorophyll, and were white or yellow in colour (Fig. 1). Several samples of red clover where a partial lack of chlorophyll prevailed were transferred, in September 1950, to pot cultures in the laboratory room, infected (Oct. 2) partly by placing *Botrytis* mycelium and conidia on the margin between the two parts of the leaflet, the green part and that without chlorophyll, partly by blowing fungal suspension all over the vegetation in the experimental pot through a fixation pipe. The plants were then covered with a glass bowl and placed on the window sill on the sunny side in the laboratory room.

These experimental plants, which were strong and had wintered at least once, proved much more resistant to disease than the dainty clover seedlings only a couple of weeks old, grown in the test tubes (cf. Table 1). None of the completely green leaflets contracted the disease, whereas both the white and yellow leaflets without chlorophyll were readily infected by *Botrytis* (Fig. 2). In the cases where the infection was established at the margin between green cells and cells without chlorophyll, the *Botrytis* mycelium spread away from the green cells, rapidly grew to the part of the leaflet without chlorophyll and killed it. The fungus spread to the green

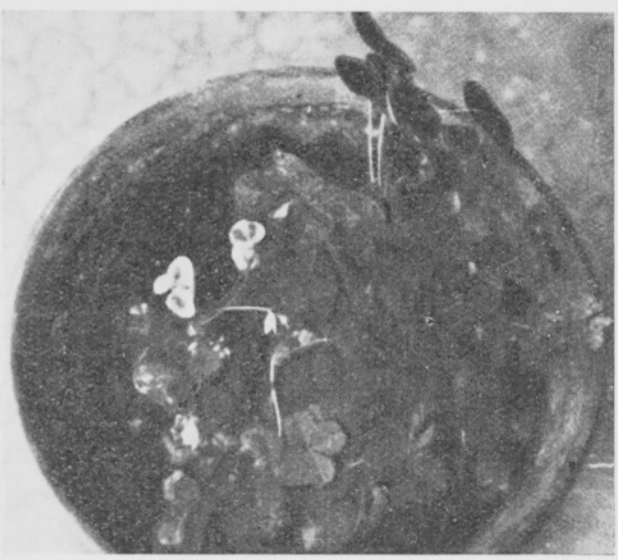

Fig. 1. Red clover leaflets entirely or partly devoid of chlorophyll (above).
Fig. 2. Red clover leaflets without chlorophyll, partly browned due to *Botrytis cinerea* (left).

cells only later, advancing as the poison excreted by it broke down the resistance of the cells (Table 3).

Effect of light on the resistance of radish (*Raphanus sativus radicula* PERS.) to *Pythium* damping-off disease.

In a series of experiments where the influence of light intensity conditions was observed on the growth of radish (variety Gaudry), plenty of *Pythium* damping-off appeared. On microscopic inspection no fungi other than *Pythium* PRINGSHEIM-*sp.* were found in the diseased seedlings. The experimental pots were placed in two laboratory rooms, and in one of them the plants received fluorescent light (approx. 1000 lux) all the time. In each room the experimental pots were placed at different distances from the window, exposed to daylight at 67, 53, 46, 38, 33 and 30 % of full daylight intensity (Fig. 3). Parallel pots totalled 3, with 15 radish seeds sown in each. The sowing was effected fairly late in the autumn (Oct. 6), and hence the radishes, even in the pots placed next to the window, received relatively little daylight.

A considerable number of the seedlings were already diseased in the germinate stage. The incidence of disease in these seedlings was not affected at all by light intensity conditions (Table 4). After shooting, distinctly the greatest number of radishes that caught the damping-off disease were those growing in the experimental pots that received no artificial light and those that were at the greatest distance from the window. As a rule the disease originated at the base of the seedlings, where there was only little or no chlorophyll. In scanty light, however, infection also appeared in the leaves.

The specimens of *Pythium* isolated from the diseased radishes in the autumn of 1950 were destroyed during the winter, and although the earth of the experimental pots was preserved the radishes sown in it in the late winter of 1951 no longer caught the damping-off disease; hence, it was unfortunately impossible to study the direct effect of light on the *Pythium* fungus employed in the experiments.

Discussion.

Red clover proved relatively resistant to *Botrytis cinerea.* In sufficient light only small seedlings were diseased. Clover seedlings grown in darkness were very rapidly destroyed by *Botrytis* (Table 1). Also older clover, resistant to disease when exposed to light, caught the disease when growing in darkness. The direct effect of light on the *Botrytis* fungus, however, was always only slight. Diffuse light had no deleterious effect whatever on the growth of its mycelium (Table 2, Feb. 28 — March 3), and even direct solar radiation perhaps weakened the growth and spore formation of *Botrytis cinerea* mycelium more by increasing the temperature and thus drying up the growth substratum than by its direct effect. It is probable, therefore, that light increased the resistance of red clover to disease. That in this case the effect of photosynthesis was fairly direct can be concluded from the results of other experiments which showed that, in adequate light, *Botrytis* did not infect the green

Fig. 3. An experiment series with Pythium damping-off, in which the pots are placed at different distances from the window of the laboratory room.

leaflets of red clover but destroyed the leaflets without chlorophyll (Fig. 2). When *Botrytis* mycelium and conidia were transferred to the margin between the green cells and cells without chlorophyll of a clover leaflet, the cells without chlorophyll were destroyed more quickly (Table 3). The green cells were not destroyed until the fungus that had spread in the cells without chlorophyll had gained in strength thanks to nutrition extracted from it, and thus was able to kill the green cells with the poison excreted. As *Botrytis* fungus did not grow from a leaflet it had destroyed to the neighbouring leaflet touching it, but its mycelium bent away from the green leaflet, it can be concluded that the resistance based on photosynthesis was spontaneous, and was not based on irritation by the parasite.

In his investigations into the damping-off of *Pinus* specifies caused by *Fusarium oxysporium,* TINT (13) came to the conclusion that the alleviating effect of light on damping-off disease was not due to photosynthesis but to the fact that light weakened the growth of the parasite and improved the succulent properties of *Pinus* species. As the present investigation, in the case of *Pythium* damping-off (Table 4), failed to clarify the immediate effect of light on the parasite, the conception of the extent to which light increased the resistance to diseases of radish remains somewhat defective. However, the fact that damping-off in intense light was restricted to the base of the seedling, nearly devoid of chlorophyll, but in scanty light infected the green leaf blade also, does not indicate that the incidence of the disease was dependent, decisively at least, on the immediate effect of light on *Pythium* fungus or on the succulent qualities of radish. It therefore seems that in this case too photosynthesis increased the resistance of the plant to disease. On the other hand, the experimental results do not show whether the increasing effect of additional light on resistance to diseases was based on intensified photosynthesis alone or whether, alongside that also factors connected with photoperiodic irritation had some influence.

Conclusions.

Lack of light increased destruction by *Botrytis cinerea* in red clover and *Pythium* damping-off in radish.

Light increased the resistance of red clover and radish to diseases, apparently by accelerating photosynthesis.

The resistance of red clover to *Botrytis cinerea* based on photosynthesis was probably spontaneous, and hence not due to irritation by the parasite.

LITERATURE.

(1) FROMME, F. D. 1915. Negative heliotropism of urediniospore germtubes. American Journ. of Botany, 2, p. 82—85.

(2) GASSNER, G. 1927. Die Frage der Rostanfälligkeit als ernährungsphysiologisches Problem. Angewandte Botanik, 9, p. 531—541.

(3) GASSNER and STRAIB, W. 1929. Untersuchungen über die Abhängigkeit des Infectionsverhaltens der Getreiderostpilze vom Kohlensäugerehalt der Luft. Phytopathologische Zeitschr. 1, p. 1—30.

(4) GASSNER, G. 1929. Experimentelle Untersuchungen über das Verhalten der Weizensorten gegen *Puccinia glumarum*. Ibid. 1, p. 215—275.

(5) GASSNER, G. 1934. Untersuchungen über das Auftreten biologischer Rassen des Weisengelbrostes im Jahre 1932. Arbeiten aus der Biologischen Reichanstalt für Land- und Forstwirtschaft, 21, p. 59—72.

(6) GÄUMANN, ERNST 1946. Pflanzliche Infektionslehre. Basel.

(7) MAINS, E. B. 1917. The relation of some rusts to the physiology their hosts. American journ. of Botany. 4, p. 179—220.

(8) POHJAKALLIO, ONNI 1932. Sokerien merkityksestä eräiden ruostesienien ravintoaineena (Über die Bedeutung der Zuckerarten als Nährungsmittel für einige Rostpilze). Acta Agralia Fennica 25.

(9) POHJAKALLIO, ONNI 1936. Sokerien vaikutuksesta kasvien ruosteenarkuuteen (Über den Einfluss der Zucker auf die Rostanfälligkeit der Pflanzen). Jour. Sci. Agricultural Soc. Finland 8, p. 89—114.

(10) POHJAKALLIO, ONNI 1947. Om orsakerna till resistens mot *Sclerotinia trifoliorum*. N. J. F:s Kongressberetning 2 (1947), p. 598—605.

(11) RODENHISER, H. A. and TAYLOR, J. W. 1942. The effect of photoperiodism on the development of bunt in two spring wheats. Phytopathology 33 (1943), p. 240—244.

(12) SEMPIO, C. 1949. Metabolic resistance to plant diseases. Ibid. 40 (1950), p. 799—819.

(13) TINT, HOWARD 1945. Studies in the Fusarium damping-off of Conifers III. Relation of temperature and sunlight to the pathogenicity of Fusarium. Ibid. 35, p. 498—510.

(14) TISCHLER, G. 1912. Untersuchungen über die Beeinflussung der *Euphorbia Cyparissias* durch *Uromyces Pisi*. Flora 104, p. 1—64.
(15) WARD, H. MARSCHAL 1905. Recent Researches on the Parasitism of Fungi. Ann. Bot. 19, p. 1—54.

SELOSTUS:

TUTKIMUKSIA FOTOSYNTEESIN MERKITYKSESTÄ KASVIEN TAUDINKESTÄVYYDESSÄ.

ONNI POHJAKALLIO, LAILA OLLILA ja KERTTU PAASI

Helsingin Yliopiston kasvipatologinen laitos.

Helsingin yliopiston kasvipatologisessa laitoksessa suoritetuissa tutkimuksissa ilmeni, että valon puute lisäsi harmaahome- (*Botrytis cinerea*-) taudin ankaruutta puna-apilassa ja *Pythium*-taimipoltteen esiintymistä retiisissä. Samalla todettiin, että valoisuus lisäsi puna-apilan ja retisiin taudinkestävyyttä fotosynteesiä kiihdyttämällä. Fotosynteesiin perustuva puna-apilan kestävyys *Botrytis cinerea*'a vastaan oli todennäköisesti spontaanista, eikä siis loisen ärsytyksestä johtunutta.
