INFLUENCE OF FATTY ACIDS ON PETUNIA POLLEN GRAINS

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SUMMARY

Fatty acids seem to be strong inhibitors of pollen germination in vitro, especially those with a carbon chain of 10 to 12 atoms. With at least one of these acids, namely 10-hydroxy-2-decenoic acid, this property is of practical importance. It prevents the germination of pollen stored by honeybees in their combs. This particular acid is added by the collecting workerbees from the mandibular glands.

10-OH-2 decenoic acid inhibits the respiration of pollen in culture solution, which could be the result of the blockage of one or more enzymes active in pollen-metabolism. In situ on the stigma, however, no inhibition of the germination occurs, probably on account of esterification with glycerides present in the stigma exudate.

1. INTRODUCTION

Pollen of higher plants is stored in high amount by bees and other insects to serve for feeding. When the pollen is brought into the hive it undergoes some changes which can be followed by the change of germination capacity (STANLEY & LINSKENS 1969). The phytocidic substance, with which the bees moisten the pollen while packing (CASTEEL 1912), seems to be a fatty acid, namely the trans-form of 10-hydroxy-2-decenoic acid ($HO\text{-}CH_2\text{-}(CH_2)_6\text{-}CH = CH\text{-}COOH$) (LUKOSCHUS & KEULARTS 1968). The fraction with free fatty acids (obtained with florisil column-chromatography) shows if esterified a decrease, and if saponified an increase of inhibiting activity. Of great importance is the question whether the fatty acid containing secretions produced by the bees during pollen-gathering, when spilled on the stigma, are harmful to the reproductive processes of the flower. Besides this it would be interesting to check the influence of fatty acids other than 10-hydroxy-2-decenoic acid on pollen germination.

2. MATERIAL AND METHODS

The two self-incompatible clones of *Petunia hybrida*, W166K (incompatibility alleles S_1, S_2) and T2U (S_3S_3) were cultivated as described before (KONAR & LINSKENS 1966a). Pollen germination-tests were carried out in the way described by LUKOSCHUS & KEULARTS (1968).

All chemicals used were of p.a. grade.

All solutions were adjusted to a level of pH 6 to 7 (with NaOH- or HCl-solution) at which pollen germination is optimal. Respiration of pollen was investigated by the Warburg method. To the main compartment was added 1 ml of pollen suspension (5 mg/ml cultivation solution), which was prepared 20 minutes before starting the experiment. In one of the side-compartments 0.5 ml of a 20 percent KOH solution and in the other 0.5 ml of the test substance solution was present. All tests were carried out at 25°C.

3. RESULTS

3.1. Influence of fatty acids on pollen germination in vitro

To find out if other fatty acids, besides 10-hydroxy-2-decenoic acid, also have an inhibiting action on the germination of *Petunia*-pollen in vitro, a series of saturated fatty acids were tested on these pollen. The results of the tests are summarized in *table 1*.

From this table, we can conclude that practically all fatty acids have an inhibiting action. The intensity of inhibition increases with the length of the carbon chain up to 10–12 atoms and then decreases again. The strongest effect is linked with 10-OH-2-decenoic acid, besides capric and lauric acid.

3.2. Influence of 10-hydroxy-2-decenoic acid on the pollen germination in situ

To check the ability of 10-hydroxy-2-decenoic acid to inhibit the pollen germination on the normal germination site of the flower, the stigma surface, 0.01 ml of a 0.01 molar solution of the sodium salt of this fatty acid was pipetted on to the stigma of 10 *Petunia* flowers and 0.01 ml distilled water on the stigma of another 10 flowers. After about 10 minutes the liquid had disappeared and a pollination with W166K pollen (T2U flowers are cross-compatible) was carried out, to find out if any seed formation would take place. After two weeks all flowers had formed mature seeds, so the pollen germination had not been inhibited. That means that the inhibiting principle, normally added to the pollen pellet by the collecting insects, has lost its activity.

3.3. Influence of 10-hydroxy-2-decenoic acid on the respiration of *Petunia* pollen

While looking for the mechanism of the action of 10-OH-2-decenoic acid, we studied its influence on pollen respiration. Respiration of pollen of *Petunia* shows in an optimal germination medium a pattern that is characterized by the short depression immediately after wetting and followed by a steady increase of oxygen uptake *(fig. 1)*. When pollen respiration is followed after addition of an extract from pollen stored in the hive, one observes depression of oxygen uptake *(fig. 2)*. This depression is strong, when extract of the mandibular glands from bees, which produce the inhibition principle (LUKOSCHUS & KEULARTS 1968), is added *(fig. 3)*. A 3.10^{-4} molar solution of the sodium salt of 10-hydroxy -2-decenoic acid, added to a pollen suspension that had been respiring for 50 minutes decreased the oxygen uptake almost immediately and even stopped it after about 2 hours. The course of the oxygen-consumption before and after addition of fatty acid solution and water, to test- and control-flask respectively, is shown in *fig. 4*.

4. DISCUSSION

It has been known for quite a long time that fatty acids have an influence on the growth of cells. WYSS *c.s.* (1945) found a fungicidal and bactericidal action

Table 1. Inhibitory effect of various fatty acids in different concentrations on the germination of *Petunia* pollen. The figures given are percentages of the germination values of the controls. Because of the low solubilities of some of these fatty acids, their concentrations could not be measured over the entire ranges presented in this table; such instances have been indicated by dashes.

Name of fatty acid	Number C-atoms	molair concentration							
		5×10^{-5}	3×10^{-4}	5×10^{-4}	10^{-3}	1.5×10^{-3}	2×10^{-3}	2.5×10^{-3}	5×10^{-3}
butyric acid	4	100	100	100	100	100	100	100	47
valeric acid	5	100	100	100	100	100	92	55	16
caproic acid	6	100	100	100	90	60	.	.	.
caprylic acid	8	100	87	45	0	0	.	.	.
capric acid	10	35	0	0	.	.	.	.	.
lauric acid	12	36	0	0	0	.	.	.	.
myristic acid	14	100	100	91	50	0	0	.	.
palmitic acid	16	100	100	100	.	.	.	.	.
stearic acid	18	100	100	100	.	.	.	.	.
10-hydroxy-2-decenoic acid	10	95	0	0	0	0	0	0	0

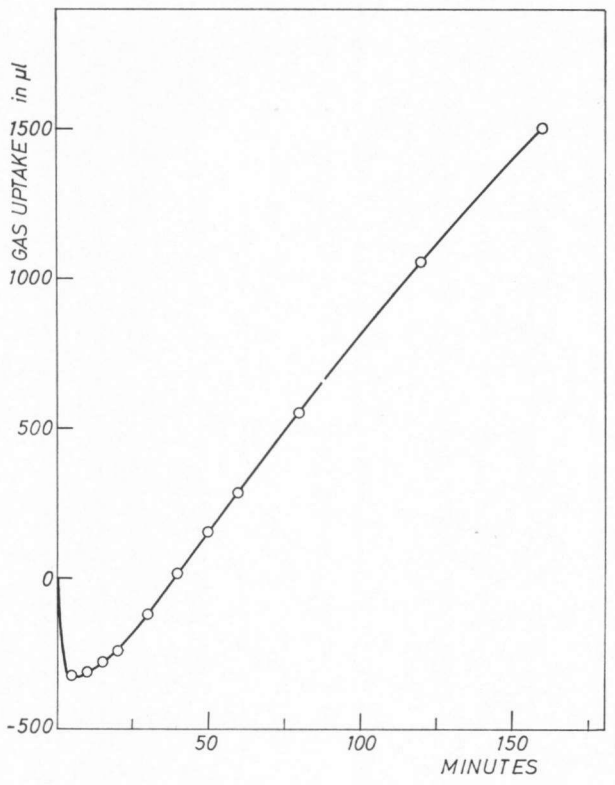

Fig. 1. Oxygen uptake of 1 ml of a suspension of 5 mg pollen of *Petunia* in 5 ml solution (10 % sucrose plus 0.05 % boric acid).

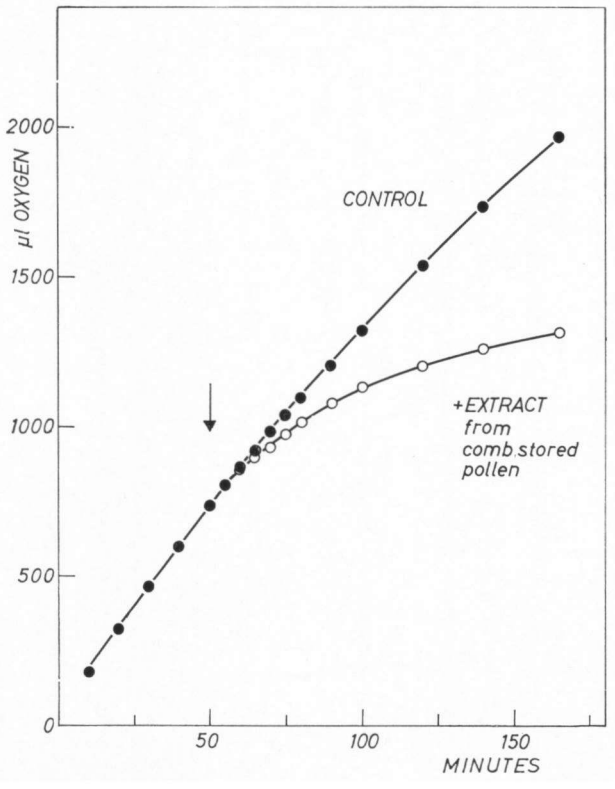

Fig. 2. Respiration of a pollen suspension (as in *fig. 1*). After 50 minutes (arrow) an extract of bee collected pollen from a comb was added.

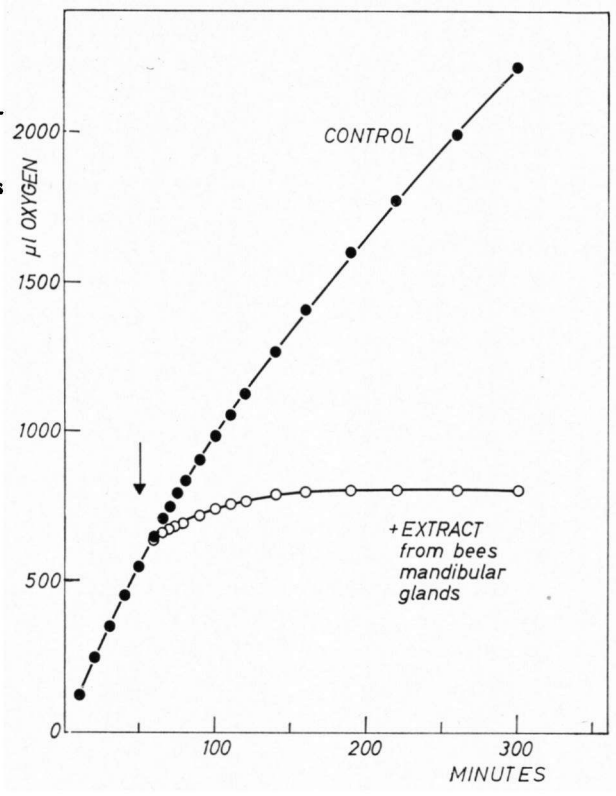

Fig. 3. Respiration of a pollen suspension (as in *fig. 1*). After 50 minutes (arrow) an extract of mandibular glands from bees was added.

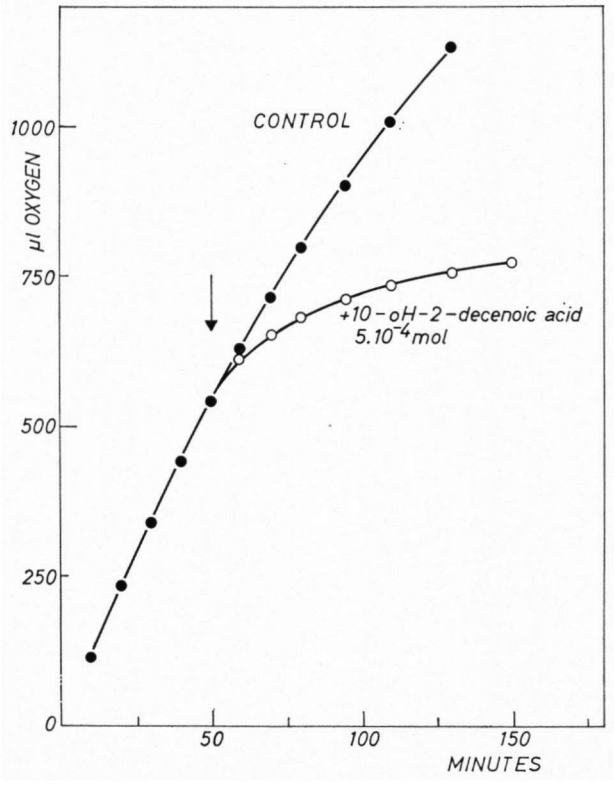

Fig. 4. Respiration of a pollen suspension (as in *fig. 1*). After 50 minutes 1 ml of a 3×10^{-4} molar solution of the Na-salt of 10-hydroxy-2-decenoic acid was added.

of fatty acids, especially those with a carbon chain of 11-atoms. ROTHMAN *c.s.* (1945) established a fungicidal action of hair fat on *Microsporon audouini* and the cause of this action also seemed to be fatty acids with 7 to 11 carbon atoms.

So our results are a fairly good application of the action of fatty acids. Besides the "pollenicidal" action of 10-hydroxy-2-decenoic acid (BUTENANDT & REMBOLD 1957), this substance also inhibits bacterial and fungal growth (BLUM *c.s.* 1959) and the expansion of artificial leukemic and ascitic tumors (TOWNSEND *c.s.* 1959).

The fact that this acid did not inhibit the pollen tube growth on the stigma and in the style of *Petunia* plants is of great importance. The explanation of this phenomenon could be esterification with glycerides of the stigma exudate of these plants (KONAR & LINSKENS 1966b), for this exudate consists of an oily-substance free of free fatty acids. So this substance could be a mixture of mono-, di- and triglycerides with many free hydroxylgroups, that could get esterified. If this was also the case with flowers that depend for their reproduction, *e.g.* pollination on insects and especially honeybees, it would be of great importance for these plants as protection against sterilization. There is no contamination of the stigma exudate with pollenicidal substances.

The effect of 10-hydroxy-2-decenoic acid on the respiration does not mean that this substance has a direct influence on this process. It is possible that one or more enzymes, active in pollen metabolism, are blocked and so prevent the course of that special reaction or reactions. Further investigation is in progress.

REFERENCES

BLUM, M. S., A. F. NOVAK & S. TABER (1959): 10-hydroxy-2-decenoic acid, an antibiotic found in royal jelly. *Science* **130**: 452–453.

BUTENANDT, A. & H. REMBOLD (1957): Über den Weichselzellenfuttersaft der Honigbiene. I. Isolierung, Konstitutionsermittlung und Vorkommen der 10-Hydroxy-2-decensäure. *Hoppe-Seyler's Z. Physiol. Chem.* **308**: 284–289.

CASTEEL, D. B. (1912): The behavior of the honeybee in pollen collecting. *Bull. U.S. Dept. Agricult. Bur. Entomol.* **121**: 36 pags.

KONAR, R. N. & H. F. LINSKENS (1966a): The morphology and anatomy of the stigma of Petunia hybrida. *Planta* **71**: 356–371.

—&—, (1966b): Physiology and biochemistry of the stigmatic fluid of Petunia hybrida. *Planta* **71**: 372–387.

LUKOSCHUS, F. S. & J. L. W. KEULARTS (1968): Eine weitere Funktion der Mandibeldrüse der Arbeiterin. Produktion eines Pollenkeimungshemmstoffes. *Z. f. Bienenforschung* (in press)

MAURIZIO, A. (1958): Pollenkeimung hemmende Stoffe im Körper der Honigbiene. *Proc. XVII. Intern. Bienenzüchter Kongr.* Bologna-Rom, 1958, p. 1–3.

ROTHMAN, S., A. M. SMILJANIC & A. L. SHAPINO (1945): Fungistatic action of hair fat on Microsporon audouini. *Proc. Soc. exper. Biol. (N.Y.)* **60**: 394–395.

STANLEY, R. G. & H. F. LINSKENS (1969): *Biochemistry of pollen.* McMillan Comp., New York (in press).

TOWNSEND, G. F., J. F. MORGAN & B. HAZLETT (1959): Activity of 10-hydroxydecenoic acid from royal jelly against experimental leukemia and ascitic tumours. *Nature* **183**: 1270–1271.

WYSS, O., B. J. LUDWIG & R. R. JOINER (1945): The fungistatic and fungicidal action of fatty acids and related compounds. *Arch. Biochem. Biophys.* **7**: 415–425.