Mean daily egg production of F_2 females during a 15-day laying period. These females were offspring of 3 F_1 sib couples, all of the same parents (P_0)

F_1 couple	F_1 characteristics Egg hatchability (%)	Egg-to-adult survival (%)	Viability* (%)	F_2 characteristics Mean daily egg production per couple N	All couples	N	Couples which laid eggs with normal development	N	Couples which laid eggs showing partial blocking	
a	69.4	86.3	59.9	37	48.8 ± 2.4	0	–	37	48.2 ± 2.4	
b	94.6	70.7	66.9	40	54.7 ± 3.6	29	57.1 ± 3.6	11	48.6 ± 9.4	$p < 0.001$
c	96.1	95.0	91.4	38	61.9 ± 4.4	30	64.0 ± 4.8	8	53.4 ± 10.5	$p < 0.001$
Controls	95.4	96.1	91.7	31	67.7 ± 1.6	31	67.7 ± 1.6	0	--	

For a, b and c F_1 couple characteristics, see text. The ensembles of F_2 couples were compared by the Mann-Whitney U test[12]. N, number of F_2 couples. * Viability is the product of egg hatchability and egg-to-adult survival of the eggs laid by the F_1 female.

but then high larvo-pupal mortality occurred. The 3rd couple (c, table) laid eggs which apparently developed normally from fertilisation to adulthood.

The F_2 adults, obtained from the above F_1 couples, were crossed between brother and sister. About 30–40 such sib couples were set up in small boxes renewed daily to determine egg production. These F_2 couples were followed throughout a 15-day laying period. Afterwards, females were dissected and their ovarioles inspected. All couples which produced unfertilised eggs were discarded since unmated females lay few eggs[8]. P_0 couples served as controls.

The table shows the F_2 average daily egg production. Production of the inbreeding sensitive a-family was smaller than that of the b- and c-families and of the controls. The b- and c-families, however, showed egg production lower than that of controls. There was considerable variation in egg production among the F_2 couples within a family. By this approach, we have been able to distinguish F_2 couples which lay eggs that may be blocked during embryogenesis from those which lay eggs that develop normally. In the couples subject to blocking, egg production was lower (table, $p < 0.001$).

These results agree with previous observations of an inbreeding effect on egg production in *Drosophila*[9,10]. However, here only certain females were responsible for the decrease. They originated from batches of inbred eggs which showed low hatchability and/or low egg-to-adult survival. A strong correlation exists between viability (table) of eggs laid by the F_1 sib couples and egg production of the F_2 offspring. Furthermore, a small egg production of the F_2 females is associated with blocking in development of some of their eggs.

Everything takes place as if lethal factors acting throughout embryonic and larvo-pupal development can also act as imaginal factors in the resulting adults. We believe these factors, which we of course observe by their physiological effects, may exert a pleiotropic effect in the ovaries of inbred adults and result in abnormalities in ovariole development. Indeed, as we observe in the present work that low egg production relates to weak vitellogenesis, non-functional or degenerated ovarioles or atrophied ovaries. Likewise, F_2 inbred females with high fecundity also have good vitellogenesis and no abnormalities.

Such effects due to brother-sister inbreeding imply that greater care is necessary in the interpretation of inbreeding depression on quantitative characters related to fitness. Indeed, inbreeding depression of egg production[9], a quantitative trait with a polygenic genetic determinism[11], is mainly due, in our opinion, to the deleterious effects observed primarily during embryogenesis.

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2 Th. Dobzhansky, B. Spassky and T. Tidwell, Genetics *48*, 361 (1963).
3 R. Greenberg and J.F. Crow, Genetics *45*, 1153 (1960).
4 N.E. Morton, J.F. Crow and H.J. Muller, Proc. natl. Acad. Sci. *42*, 855 (1956).
5 R.C. Lewontin, in: The genetic basis of evolutionary change. Columbia University Press, New York, London 1974.
6 C. Biémont, Mech. Age Devt *8*, 21 (1978).
7 J. David, Bull. Biol. Fr. Belg. *93*, 472 (1959).
8 J. Bouletreau-Merle, Thesis 7516, Lyon 1975.
9 D. Marinkovic, Genetics *56*, 61 (1967).
10 C. Biémont, C.r. hebd. séanc. Acad. Sci. Paris *278*, 1095 (1974).
11 J.F. Kidwell, Can. J. Genet. Cytol. *11*, 547 (1969).
12 S. Siegel, in: Nonparametric statistics. McGraw-Hill, New York, Toronto, London 1956.

Inhibition of the regulatory ability of stomata caused by exhaust gases

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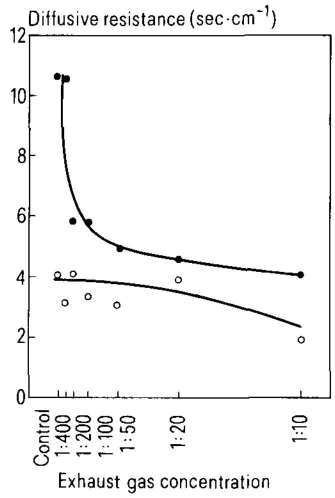
Summary. It is demonstrated that very low concentrations of exhaust gases from a combustion engine inhibit the regulatory ability of stomata. However, when gas treatment was stopped, plants showed a quick recovery of the ability to close stomata.

The stomata represent the pathway to the gas exchange for the green plant. One of the factors that influences the extent of the opening of the stomata is the prevailing CO_2-concentration in the environment. Low CO_2-concentrations result in the opening of the stomata[2], while high concentration causes their closing[3,4]. It would therefore be expected that the presence of exhaust gases from combustion engines, with their high content of CO_2, should cause the closing of the stomata. But, infact, investigations performed on aspen have shown that exhaust gases induce an opening of stomata.

Pottet clones of young aspen (*Populus tremula*) were exposed to 30 klux, 25 °C, and 65% relative humidity in chambers through which exhaust gases of different concentrations flowed from a gasoline generator. After a period of 2 h exposure to light, as well as additional 2 h to darkness, measurements of stomatal diffusive resistance were taken by means of a porometer. The air for the control chamber and the rarefying air for the individual concentration of exhaust gas were filtered through charcoal. The relationship between clean air and exhaust gas on the one hand, and the flowing through of gas on the other, were carefully controlled by means of a flow meter. Sample tests were taken to determine the amount of CO_2, CO, HC, and NO_x.

As can be seen from the figure, plants show a low stomatal diffusive resistance at light exposure, that is opened stomata, under all conditions of exhaust gas concentrations. In spite of the increased CO_2-concentration, no closing of the stomata can be observed. On the contrary, it can be seen that, with the high exhaust gas concentration of 1:10, the resistance, as compared to the control plant, is significantly lower. During the period of darkness, tests showed the usual high stomatal diffusive resistance, whereas it could be observed that already a high degree of gas exhaust rarefaction of 1:200 causes the stomata to remain open. It therefore appears that the regulatory ability of the stomata is inhibited by certain exhaust gas components. It is known that SO_2 has such an effect[5-7]; however, in conducting these experiments, SO_2, because of its low concentration in exhaust gas, could hardly have had a significant influence upon the regulatory ability.

Field experiments in the vicinity of a motorway confirm the possibility that exhaust gas concentrations in heavy traffic situations may be sufficient to exert an influence on the stomata apparatus. Plants growing immediately along the motorway, as compared to plants growing at a distance of 200 m from it, show lower stomatal diffusive resistance during the hot noon hours, when normally a closure of stomata takes place. However, examinations under the microscope have pointed out that in plants growing along the motorway the closing of stomata is inhibited by particles of dust as well. Additional inhibiting effects upon the stomata, such as these, could further contribute to permanently open stomata. These observations are supported by laboratory tests, pointing out that shortly after exposure to gases, but in the absence of the effect of dust particles, the stomata soon showed a regeneration of its regulatory ability. When plants were subjected to a 4-h exposure to 1:200 exhaust gas in the dark, and subsequently to clean air for a period of 30 min, a significant increase was noted in the stomatal diffusive resistance from 54% in the exhaust gas atmosphere to 66% in clean air, as compared to 100% of the control plants. As a consequence of this failure of stomatal regulation, the water economy in hot and dry weather conditions can be damaged by excessive transpiration.



Stomatal diffusive resistance of clones of *Populus tremula* in dependence of various exhaust gas-air mixtures; ○, in light, ●, in darkness. The resistance is significantly lower in light between control (clean air) and gas mixture 1:10 (2 $p < 0.001$), and in darkness between control and gas mixture 1:200 (2 $p < 0.001$). $n = 8$. Exhaust gas concentrations at 1:100:CO_2 600 ppm, CO 40 ppm, NO_x 0.05 ppm, hydrocarbons (hexene aequivalent) 1 ppm.

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2 I. Frommhold, Biochem. Physiol. Pflanzen *163*, 216 (1972).
3 O.V. Heath and J. Russel, J. exp. Bot. *5*, 269 (1959).
4 J.E. Pallas, Jr, Science *147*, 171 (1965).
5 W. Koch, Planta *48*, 418 (1957).
6 O. Majernik and T.A. Mansfield, Nature *227*, 377 (1970).
7 O. Majernik and T.A. Mansfield, Environ. Pollut. *3*, 1 (1972).
8 T.T. Lee, Can. J. Bot *43*, 677 (1965).

Voltage-clamp analysis of the sodium and potassium currents in skeletal muscle fibres treated with 4-aminopyridine[1]

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Summary. External application of low concentrations of 4-aminopyridine blocks potassium currents without affecting sodium currents in pieces of single frog skeletal muscle fibres. The blockade of potassium currents was voltage-dependent, being partially relieved on depolarization.

Among the compounds that interfer with the operation of the potassium channels in excitable membranes, tetraethylammonium (TEA) and its derivatives are the best known[3-6]. Recent studies performed with aminopyridines have shown that 2-, 3-, and 4-aminopyridine block the potassium channels in a variety of nerve membranes[7-12]. 4-aminopyridine (4-AP) is known to prolong the repolarizing phase of the action potential of frog and rat skeletal muscle fibres[13-15], and has been shown to inhibit the potassium conductance in frog muscle fibres in lower concentrations than does TEA[16]. The present experiments examine in more detail 4-AP's action on sodium and potassium currents in fragments of single frog skeletal muscle fibres bathed in a normal ionic medium, under voltage-clamp conditions, in order further to characterize its mode of action.

Methods. Experiments were performed on pieces of single muscle fibres (having a diameter of 90–150 μm and a length