Proc. Natl. Acad. Sci. USA
Vol. 77, No. 10, pp. 5631-5633, October 1980
Applied Biology

Ambient air concentration of sulfur dioxide affects flight activity in bees

(air pollution/insects)

M. E. GINEVAN*[†], D. D. LANE[‡][§], AND L. GREENBERG*

Departments of *Entomology and [‡]Civil Engineering, University of Kansas, Lawrence, Kansas 66045

Communicated by Charles D. Michener, July 25, 1980

ABSTRACT Three long-term (16-29 days) low-level (0.14-0.28 ppm) sulfur dioxide fumigations showed that exposure to this gas has deleterious effects on male sweat bees (*Lasioglossum zephrum*). Although effects on mortality were equivocal, flight activity was definitely reduced. Because flight is necessary for successful mating behavior, the results suggest that sulfur dioxide air pollution could adversely affect this and doubtless other terrestrial insects.

Sulfur dioxide is one of the major atmospheric contaminants associated with the combustion of fossil fuels. Several recent studies suggest that low ambient concentrations of this gas may adversely affect terrestrial insects. The first such study (1) showed that a 14-week fumigation of honey bee (*Apis mellifera*) colonies at a sulfur dioxide concentration of less than 0.5 ppm significantly reduced honey bee foraging activity and total colony weight gain. Other investigations involving the fruit fly (*Drosophila melanogaster*) demonstrated that an 11-day fumigation at a sulfur dioxide concentration of 0.4 ppm drastically reduced larval feeding activity and thus nearly doubled developmental time; it was also shown that a 4-day exposure at 0.4 ppm significantly reduced pupal survival and that an exposure of several weeks at the same concentration reduced adult longevity (2, 3). Controlled fumigations of field plots indicate that sulfur dioxide concentrations of less than 0.1 ppm significantly reduce the abundance of certain beetle species (*Canthon* sp.) as measured by pitfall trap captures (4).

These experimental studies are supported by field observations. Several workers noted elevated populations of herbivorous insects and reduced abundance of the insect parasitoids normally associated with these herbivores in areas subject to sulfur dioxide stress (1, 5, 6). This suggests that sulfur dioxide gas upsets host-parasite relationships.

In two of the species mentioned above (*D. melanogaster* and *A. mellifera*), sulfur dioxide exposure was shown to reduce activity levels (1-3), and the hypothesis that exposure to low levels of sulfur dioxide reduces insect activity may account for the other observations as well. In the case of the beetles (4), it may be that less-active beetles fall into pitfall traps less often, thereby creating an apparent reduction in abundance. Likewise, insect parasitoids that exert effective biological control on their host species keep both themselves and the host species at low abundance. Thus, female parasitoids must vigorously search out hosts, and reduced activity of the parasitoid results in lower parasitoid reproductive success coupled with increased survival of the host species (7).

Males of the sweat bee species *Lasioglossum zephyrum* spend the majority of the day in flight (8). This activity is vital to mating success. Since many flying insects show respiratory rates 100 times the resting level (9), they should be vulnerable to airborne toxins. This, coupled with the fact that reductions in the normally high flight activity levels of *Lasioglossum* males would be readily apparent, formed the basis of the present study.

EXPERIMENTAL PROCEDURE

Details of the construction and operation of our exposure apparatus are given elsewhere (10). All experiments were carried out at 28°C in 70% relative humidity with 16-hr light/8-hr dark cycle. Sulfur dioxide concentrations were monitored continuously with a Meloy Laboratories flame photometric sulfur hydrocarbon analyzer (model SH 202) and at all times were within 5% of the nominal treatment concentrations. During the experiments, small sponges moistened daily with a mixture of 1 part honey and 5 parts water were provided as food source for the bees.

In our first experiment, control and experimental groups of 10 male bees 10-21 days old were used. Flight activity was determined by one 10-min observation period per day. Twenty observations, taken at 30-sec intervals, of the number of bees flying in the experimental and control groups were made. Percentage flight activity for the experimental and control groups was then determined by the formula: $P_f = [F/(20\,N)] \times 100$ in which P_f is the percent flight activity, F is the total number of flying bees observed, and N is the total number of bees alive (thus, $20\,N$ is the maximum possible number of flight observations). Both groups were observed for 3 days prior to the onset of fumigation to ensure comparable flight activity levels. During this period both groups were maintained in filtered air. Thereafter, the experimental group was continuously exposed to a sulfur dioxide concentration of 0.14 ppm; the controls were maintained in filtered air. Daily observations of flight activity were continued until fewer than five bees were alive in one group. All observations of flight activity were made between 1330 and 1500 CDT.

The second and third experiments followed the methods of experiment 1, with the following exceptions. In experiment 2, 11 bees 7-14 days old were used in both experimental and control groups. In experiment 3, 12 bees 7-14 days old constituted the experimental and control groups, and fumigation with sulfur dioxide was carried out at 0.28 ppm. The small differences in number and ages of the bees used were dictated by limited availability of male bees.

In all experiments, change in flight activity was measured by a least-squares regression of percentage flight activity against time measured in days. In each experiment, differences in flight activity between experimentals and controls were tested by a *t* test for the equality of regression coefficients (11).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

[†] Present address: Division of Biological & Medical Research, Argonne National Laboratory, Argonne, IL 60439.

[§] To whom reprint requests should be addressed.

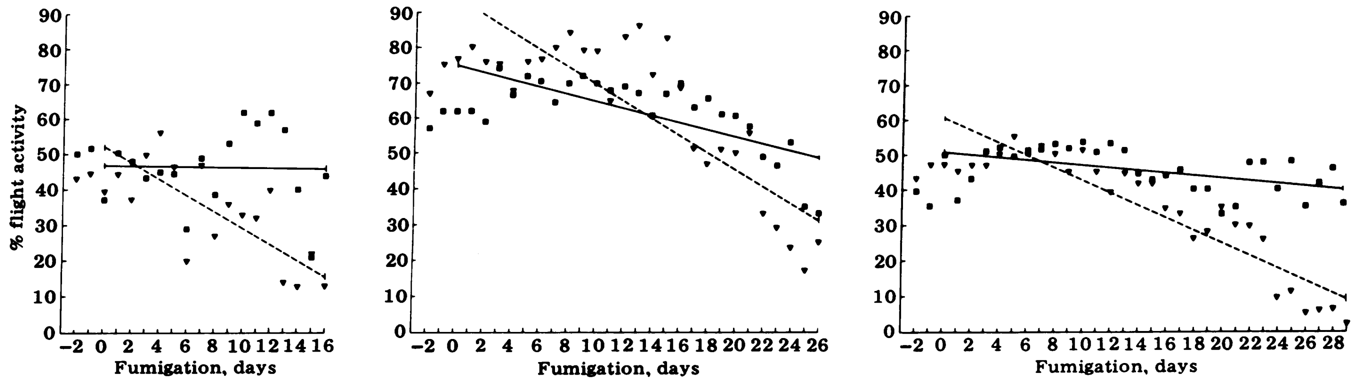

FIG. 1. Plots of percentage flight activity against time of fumigation with the associated regression lines. ▼, Experimental (- - -); ■, control (——). (*Left*) Experiment 1, SO_2 at 0.14 ppm, 16 days; (*Center*) experiment 2, SO_2 at 0.14 ppm, 26 days; (*Right*) experiment 3, SO_2 at 0.28 ppm, 29 days.

RESULTS AND DISCUSSION

Plots of percentage flight activity versus time for the three experiments are shown in Fig. 1. There were some obvious differences among the experiments. Most notable of these were that the general flight activity level of the bees was much higher in experiment 2 than in experiment 1 or 3 and that longevity patterns (Table 1) were heterogeneous among the three experiments. Nonetheless, there were a number of features common to all experiments. Flight activity decreased more rapidly in the experimental bees than in the controls in all experiments (Fig. 1; Table 2). Further, the rate of decrease in flight activity as measured by the magnitude of the regression coefficient was similar for all three groups of experimental bees (Table 2).

Table 1. Number of bees surviving over time in experiments 1–3

	Experiment 1		Experiment 2		Experiment 3	
Day	E	C	E	C	E	C
−2	10	10	11	11	12	12
−1	10	10	11	11	12	12
0	10	10	11	11	12	12
1	10	10	11	11	12	12
2	9	9	11	11	12	12
3	9	8	11	11	12	11
4	9	8	11	11	12	11
5	9	8	10	11	12	11
6	9	7	10	11	12	11
7	9	6	10	11	11	11
8	9	5	10	11	11	10
9	9	5	10	11	11	10
10	9	5	9	11	11	10
11	9	5	9	11	11	9
12	9	5	9	11	11	9
13	9	5	9	11	11	9
14	8	5	9	11	11	8
15	8	4	9	11	11	8
16			9	11	11	8
17			9	11	10	7
18			9	10	9	7
19			8	10	9	6
20			8	10	9	6
21			8	10	9	6
22			7	10	9	6
23			7	9	8	6
24			5	9	7	6
25			4	9	7	6
26					5	5
27					5	5
28					4	5

Experiments 1 and 2 were at $SO_2 = 0.14$ ppm; experiment 3 was at $SO_2 = 0.28$ ppm. E, experimental; C, control.

Because of varying ages of the bees it might be expected that longevity would be a relatively poor indicator of sulfur dioxide effect (although in two of the three experiments, more control than experimental bees were alive at the termination of fumigation). Furthermore, in insects as active as *Lasioglossum* males, a sublethal toxic agent which suppressed activity, and thus greatly reduced metabolic rate, could well have paradoxical effects on longevity in a laboratory setting. The point is that the activity patterns observed are consistent and obviously independent of mortality patterns. Thus, they provide evidence that fairly low concentrations of sulfur dioxide can reduce insect activity levels.

The gradual reduction in flight activity suggests a physiological as opposed to a behavioral basis. The presumed mechanism is accelerated deterioration of the flight muscles. This hypothesis is supported by the fact that, at the end of each experiment, the experimental bees could walk normally but were able to fly only weakly, if at all. By contrast, considerable care had to be exercised in removing the control bees from their chamber lest they escape by flying away.

These observations provided a probable mechanism for the decline in foraging behavior observed in fumigated honeybees (1) and at least a possible mechanism for the apparent reduction in success of insect parasitoids (1, 5, 6), since in either case reduced flight ability would produce the observed effect.[¶]

The observed changes in *Lasioglossum* flight activity are less relevant to the observed changes in larval feeding behavior of *Drosophila* (2, 3) or to the reduced pitfall captures of ground beetles (4). In the case of *Drosophila* a different life stage was involved and the underlying mechanism is almost certainly behavioral (3). This is reasonable in that *Drosophila* larvae are known to be able to modify their developmental rate in response to other stresses (12). Furthermore, the larval stage inhabits rotting vegetable material. Thus, it would be liable to exposure to noxious gases resulting from the decay process and might have evolved responses to such toxins. It is interesting to note that the beetles described in ref. 4 also feed on decaying organic matter. Although the data are sparse, the responses of *Drosophila* might be most relevant to their behavior.

[¶] In the case of honeybees, only hives were fumigated, but the concentrations used were 3 times those used here, and considerable "flight" in the form of ventilation activities takes place within the hives.

Table 2. Summary of regressions of flight activity against time

	SO_2,	Experimental					Control					
Exp.	ppm	a	b	r	t	df	a	b	r	t	df	$t(b_e - b_c)$
1	0.14	51.98	−2.23	−0.761	4.39*	14	46.79	−0.05	−0.021	0.07	14	2.68†
2	0.14	93.69	−2.34	−0.831	7.33*	24	75.36	−1.00	−0.716	5.02*	24	3.56*
3	0.28	60.70	−1.78	−0.917	11.96*	27	50.96	−0.37	−0.502	5.59*	27	7.28*

For each regression, the values for the intercept (a), correlation coefficient (r), its associated t statistic (t), and the number of degrees of freedom (df) are given. The last column gives the t statistic for the comparison of the slope of the experiment regression with the slope of the control regression.

* $P < 0.001$.

† $P < 0.02$.

CONCLUSIONS

The data presented suggest that real-world sulfur dioxide air pollution affects insect communities. The national 24-hr primary air quality standard for sulfur dioxide is 0.14 ppm (13) and violations of this standard are not unknown. Doubling the exposure concentration to 0.28 ppm did not markedly increase the deleterious effects observed, which suggests that the dose–response relationship is relatively flat. Thus, it seems probable that sulfur dioxide concentrations of somewhat less than 0.14 ppm should produce measurable effects, as was shown in one earlier study (4). Also, experiments that examine insect mortality may give an erroneous view of the effect of sulfur dioxide on insect populations. The reduced flight activity of *Lasioglossum* males would certainly be of significance in wild populations because males that do not maintain a high flight level do not mate successfully.

Finally, it should be pointed out that, until recently, green plants have provided the major indicator organisms for air pollution stress (14). It would seem probable that some insect systems (particularly those which consider behavioral end points) could serve a similar purpose.

The bees were available for this study because of National Science Foundation Grant BNS 78-07709 (C. D. Michener, principal investigator) for behavioral studies of these insects. Also, we acknowledge the support of the Department of Civil Engineering in the form of equipment and laboratory supplies.

1. Hillman, R. C. (1972) Dissertation (Pennsylvania State University, University Park, PA).
2. Ginevan, M. E. (1976) *Potential Effects of Sulfur Dioxide Air Pollution on Insects*, Center for Research Incorporated Env. Res. Lab. Tech. Rep. No. 7602 (Univ. of Kansas, Lawrence, KS).
3. Ginevan, M. E. & Lane, D. D. (1978) *Environ. Sci. Technol.* **12,** 828–831.
4. Bromenshenk, J. J. (1978) *The Bioenvironmental Impact of a Coal-fired Power Plant,* Third Interim Report Colstrip, MT, EPA Report No. 600/3-78-021 (NTIS, Washington, DC), p. 473.
5. Heagle, A. S. (1973) *Annu. Rev. Phytopathol.* **11,** 365–368.
6. Przybylski, A. (1967) *Postepy Nauk Rola.* **2,** 111–113.
7. Varley, G. C., Gradwell, G. R. & Hassel, M. P. (1974) *Insect Population Ecology, An Analytical Approach* (Univ. of California Press, Berkeley, CA).
8. Barrows, E. M. (1976) *J. Kans. Entomol. Soc.* **49,** 105–108.
9. Chapman, R. F. (1971) *The Insects Structure and Function* (American Elsevier, New York).
10. Ginevan, M. E. & Lane, D. D. (1980) *Econ. Entomol.,* **73,** 46–48.
11. Sokal, R. R. & Rohlf, F. J. (1969) *Biometry* (Freeman, San Francisco).
12. Budnik, M. & Brncic, D. (1974) *Ecology* **55,** 657–661.
13. U.S. Federal Register 36 (1971) (GPO, Washington, D.C.), No. 84, Part II, p. 8186.
14. U.S. Environmental Protection Agency (1973) *Effects of Sulfur Oxides in the Atmosphere on Vegetation,* Publ. R3-73-030 (GPO, Washington, D.C.).