METHOD FOR CONTINUOUSLY MONITORING THE CO_2 OUTPUT OF CAGED INSECTS

Potential Applications in Quality Control of Colonized Insects

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METHOD FOR CONTINUOUSLY MONITORING THE CO_2 OUTPUT OF CAGED INSECTS

Potential Applications in Quality Control of Colonized Insects

By W. K. Turner, N. C. Leppla, R. H. Guy, and F. L. Lee[1]

ABSTRACT

The CO_2 monitoring system incorporates elements that regulate, filter, hydrate, and control the temperature of air supplied to an insect cage in a bioclimatic chamber. The amount of CO_2 in air discharged from the cage is continuously measured by an infrared gas analyzer. This CO_2 output is directly related to the physical activity of confined insects and, therefore, indicates potentially significant behavioral differences between test populations. In quality control research, for example, wild adult cabbage loopers, *Trichoplusia ni* (Hübner), produced considerably more CO_2 than the laboratory strain. Thus, the colonization process selected inadvertently for increased docility. **KEYWORDS: CO_2 production, infrared gas analyzer, insect colonization, quality control.**

INTRODUCTION

Since both physical and metabolic aspects of behavior involve respiration, carbon dioxide output provides a convenient index of the overt activity of insect populations (*8*).[2] Furthermore, respiration rates can be measured directly by infrared gas analysis, without performing intermediate volumetric determinations or interpreting mechanical transductions (*2, 5, 9, 11*). This approach is reasonably uncomplicated; the equipment is compact and inexpensive, and the resulting measurements are exceptionally quantitative, objective, reproducible, and accurate.

The application of electronic instruments for analysis of insect behavior is justified by the extraordinary effort that is being expended to use parasites, predators, and physically or genetically altered species to manage populations of pest insects. The purpose of these strategies is to prevent the multiplication of pests by eliminating reproductive individuals or by effecting unproductive matings. Accomplishment of this objective will depend on development of technology for efficiently colonizing appropriate insects and insuring their behavioral adequacy.

This paper describes a method for continuously monitoring the CO_2 output of caged insects. The quantity and periodicity of CO_2 produced by a colonized strain of adult cabbage loopers, *Trichoplusia ni* (Hübner), is compared with that of a caged wild population.

CO_2 MONITORING SYSTEM

The simplified CO_2 monitoring system is composed of devices that regulate, filter, hydrate, heat, and monitor air that circulates

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[2] Italic numbers in parentheses refer to items in "Literature Cited" at the end of this publication.

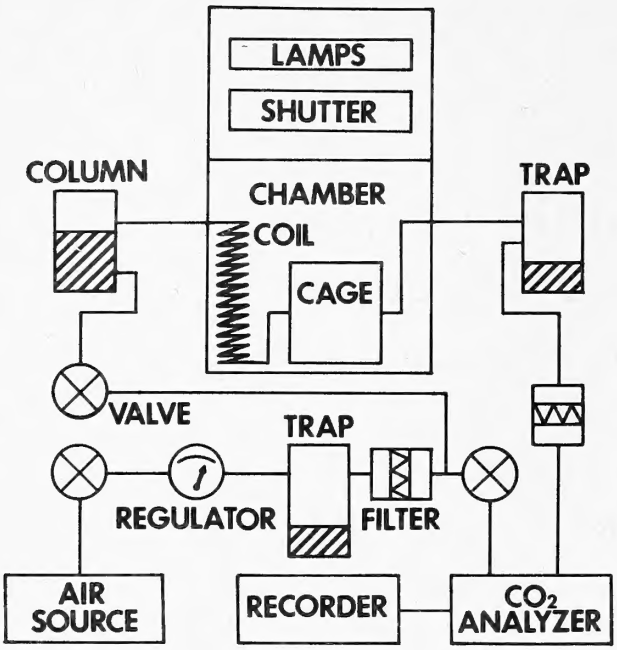

FIGURE 1.—Elements of CO_2 monitoring system.

through an insect cage maintained in a bioclimatic chamber (fig. 1). Air provided by a compressor must be filtered for particles and vapors. However, only a low-pressure regulator and needle valve are required if CO_2-free bottled air is used. Air from either source is passed through a water column and heated to 27° ± 0.5° C in a 400- by 1.2-cm-diameter heat exchange coil after entering the chamber. CO_2-laden air from the sealed cage is refiltered to remove moth scales and other contaminants before it enters a Mine Safety Corp. model 200 infrared gas analyzer. The analyzer output voltage is printed as a continuous line (1-minute response time) on a strip chart. However, since resulting voltage curves are not proportional to the CO_2 content of the gas, several point-by-point conversions must be made to determine the average quantity of CO_2 produced by an insect during each hour.

The 758-liter test chamber is a plywood box covered with Celotex on the internal and external surfaces to insure thermal and sound insulation (fig. 2A). This enclosure provides ambient temperature, relative humidity, and light for the 12-liter insect cage. This cubical cage, fabricated from 6-mm-thick Plexiglas, has air-inlet and air-outlet ports in the center of two opposing sides. It also has a 3-mm-thick glass lid that transmits 80% to 90% of the incident radiation of 350 nm and longer wavelengths (*13*). The lid is held in place with springs, and insects are introduced through a 3.8-cm-diameter stoppered hole near the top of the cage.

Light, supplied by four 20-W General Electric Cool White fluorescent lamps suspended near the ceiling of the test chamber, diffuses uniformly through a plastic partition (fig. 2B). A Gamma Scientific model 700 spectral radiometer is used to determine spectral outputs, and a Hewlett Packard model 833 A radiant flux meter with a model 8334 A detector head is used to calibrate light levels within the insect cage. Photoperiod is cycled gradually by a cam-operated shutter driven by a 24-hour clock (fig. 2C). The eccentric cam controls a piston that forces a mechanical lever to adjust the shutter openings. The spring-loaded shutter mechanism automatically closes the openings as pressure is released from the piston. Thus, spectral distributions remain constant as the light intensity gradually fluctuates during photophase and scotophase transitions. Temperature and RH are maintained in the cage by placing the chamber in a relatively cool room (21° C) and ducting in hot air from a box that contains a heater element and fan. A thermistor in the return duct connects with a solid-state temperature controller that actuates the heater.

A procedure was established for checking the CO_2 monitoring system and insects and for initiating, conducting, and terminating each test. At the beginning of each test, the equipment is cleaned, the cage is lined with white paper toweling, the photoperiod clock and cams are inspected, environmental monitoring instruments are serviced (temperature, RH, light, and time), and the airflow rate is calibrated. Next, the electrical circuits, CO_2 detector, and recorder are checked and actuated. Bottled gas of known CO_2 content is used to calibrate the detector.

The insects are maintained during the pupal stage and for 48 hours after emergence under conditions identical to the test environment. Then these preconditioned insects are transferred to the provisioned cage with an aspirator (*10*). Optimum results are obtained by using 100 adults (from about 200 mg pupae) and an air-exchange rate of 600 cm^3/min, or a similar proportion. Populations are monitored continuously, and data are analyzed manually or by

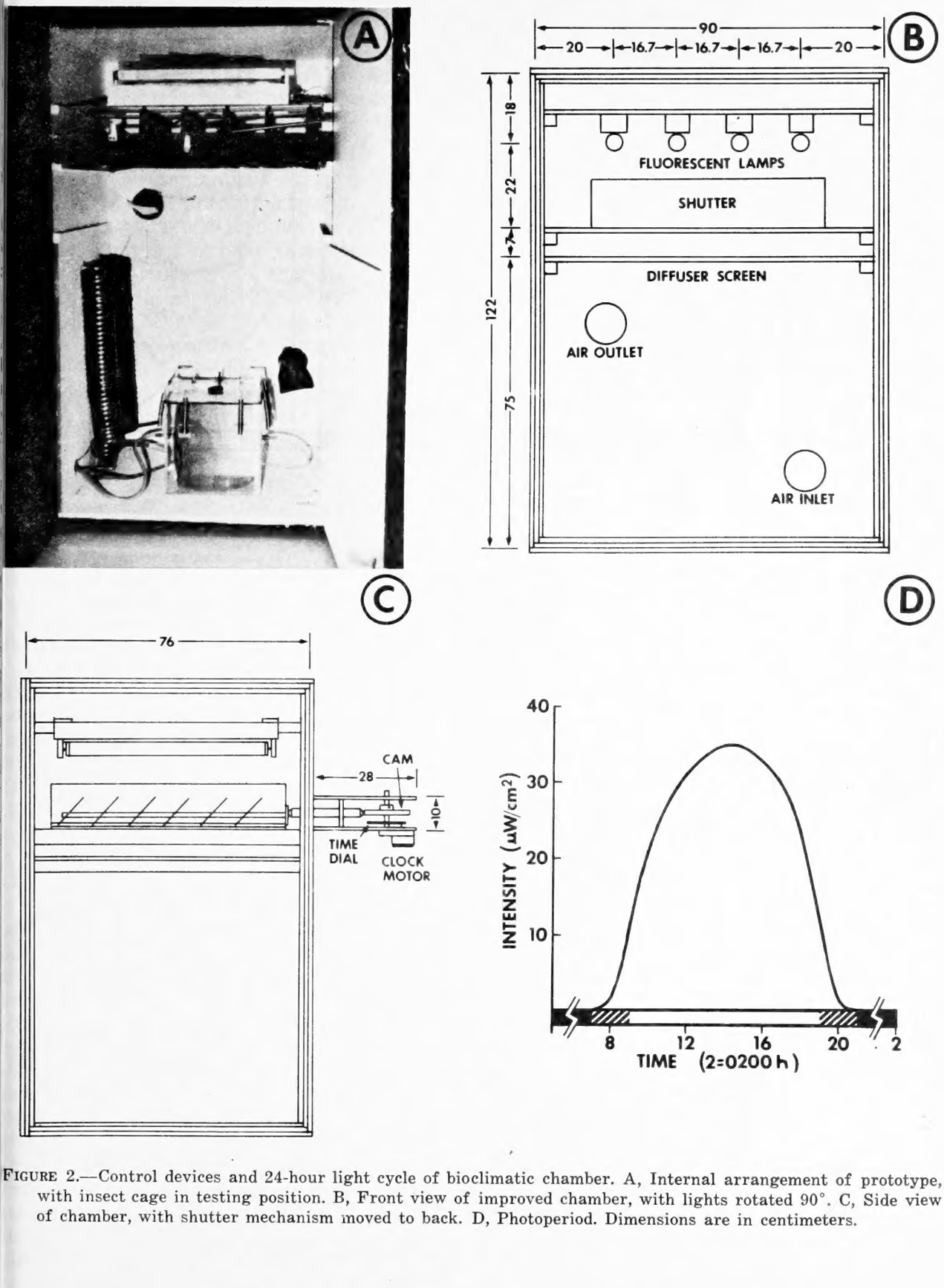

FIGURE 2.—Control devices and 24-hour light cycle of bioclimatic chamber. A, Internal arrangement of prototype, with insect cage in testing position. B, Front view of improved chamber, with lights rotated 90°. C, Side view of chamber, with shutter mechanism moved to back. D, Photoperiod. Dimensions are in centimeters.

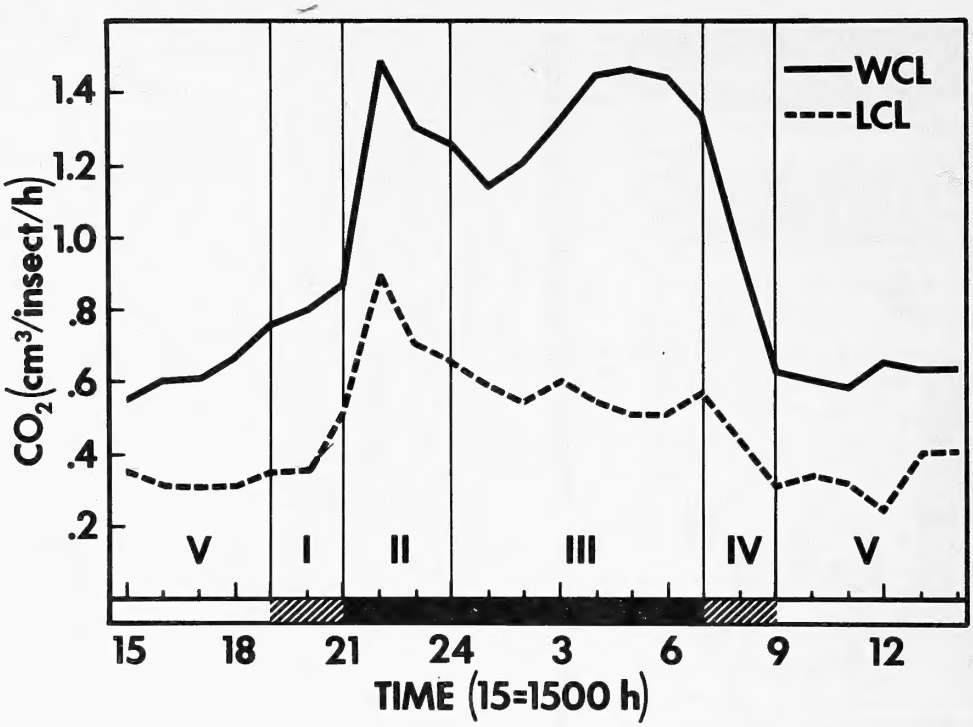

FIGURE 3.—Average hourly quantity of CO_2 produced by wild (WCL) and laboratory-reared (LCL) cabbage looper moths, 15 males and 30 females per test, during 5 time intervals of each 24-hour period (2 replications).

computer (*12*). Records are kept on the source, species, stage, sex, number, and age of the insects. Tests are replicated as is statistically appropriate for each species (*7*).

APPLICATION OF METHOD IN QUALITY CONTROL

The relative quality of wild (WCL) and laboratory-reared (LCL) cabbage loopers was evaluated by monitoring the CO_2 production of 2-day-old adults. The LCL colony was established in 1971 with about 50 pairs of moths from the same field locality as the WCL. For the comparison, 15 males and 30 females of each population were transferred successively by strain to the 12-liter cage of the CO_2 monitoring system. Temperature, RH, and photoperiod (12 hours of light from 0800 to 2000) were cycled gradually to achieve diurnal conditions of 27°±1° C, 60%±2% RH, and 35 μW/cm^2 and nocturnal conditions of 24°±1° C, 78%±2% RH, and 0.35 μW/cm^2 (fig. 2D). Tests were conducted for 72 consecutive hours, and data from the second 24 hours were used for the analysis.

Nocturnal activity, indicated by the CO_2 output of both WCL and LCL, was initiated during time interval I (1900 to 2100), but LCL responded at higher light intensities, i.e., earlier in the scotophase (fig. 3). LCL had a unimodal periodicity, whereas WCL produced a second peak during time interval III (2400 to 0700). Also, LCL yielded less than half as much CO_2 per insect during the entire test period (LCL=10.62 cm^3, WCL=22.97 cm^3). A similar proportionality was apparent for time intervals I, II (2100 to 2400), IV (0700 to 0900), and V (0900 to 1900).

These results indicate potentially significant behavioral differences between WCL and LCL that were caused by adaptation of LCL during colonization. They do not, however, identify specific performance criteria or capabilities. Assessments of the relative quality of insect populations depend on the purpose for which the insects are produced (*3*). If the WCL traits of lower thresholds of response to light intensity and increased metabolic activity during the reproductive interval are essential to the successful performance of desired functions, LCL could be of relatively low or substandard quality. Functions of this nature would include dispersal in the field, host-seeking capabilities,

mating competitiveness with the field population, and related activities (*1, 6*). Conversely, LCL have a lower metabolic rate and increased fecundity, fertility, and survival to reproductive age in the insectary. This is an advantage for producing more biomass per unit of invested resources.

Continual determinations of the periodicity and quantity of CO_2 produced by representative samples can be used to monitor the stability of colonies. However, extreme variations indicate only potentially deleterious changes and do not identify specific causes. Therefore, this technique should be used in concert with other independent assessments. Its application ultimately depends on the identification of associated behavioral traits and the preservation of desired capabilities in colonized strains.

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