HSV in the monkey kidney cells, the number of cells with both types of virus particles within the same nucleus was smaller than that observed previously in similar cultures infected with SV40 and adenovirus 12 (*1*). Titrations of HSV in the progeny of cultures infected with HSV and SV40 showed no enhancement of HSV similar to the enhancement of adenovirus 12 by SV40 (*4*). Nevertheless, the morphological demonstration of HSV and SV40 in close proximity within the nucleus of the same cell suggests the possibility of phenotypic mixing, with production of virus particles having altered biological properties.

ALAN S. RABSON, GREGORY T. O'CONOR
FRANCES J. PAUL, IRENE K. BEREZESKY
Pathologic Anatomy Branch, National Cancer Institute, Bethesda, Maryland

References

1. G. T. O'Conor, A. S. Rabson, I. K. Berezesky, F. J. Paul, *J. Nat. Cancer Inst.* **31**, 903 (1964).
2. R. J. Huebner, R. M. Chanock, B. A. Rubin, M. J. Casey, *Proc. Nat. Acad. Sci. U.S.* **52**, 1333 (1964); W. P. Rowe and S. G. Baum, *ibid.*, p. 1340; F. Rapp, J. L. Melnick, J. S. Butel, T. Kitahara, *ibid.*, p. 1348; J. M. Easton and C. W. Hiatt, *Proc. Nat. Acad. Sci. U.S.* **54**, 1100 (1965).
3. W. K. Ashe and H. W. Scherp, *J. Immunol.* **91**, 658 (1963).
4. A. S. Rabson, G. T. O'Conor, I. K. Berezesky, F. J. Paul, *Proc. Soc. Exp. Biol. Med.* **116**, 187 (1964).

21 December 1965

Population Flushing with Sexually Sterile Insects

Abstract. *In theory, populations of animals can be displaced by overloading a resource with introduced sterile animals. The theory was tested on natural populations of the Queensland fruitfly* Dacus tryoni *Frogg. Three of four treated populations declined sharply within 2 days after sterile flies had been introduced. This procedure may be useful both as a tool in experimental ecology and as a means of controlling pests.*

The idea of using artificially reared sterile animals to drive out and replace the fertile members of a natural population ("population flushing") was earlier proposed (*1*). The model predicted that fertile animals could be killed or forced to leave a population by introduction of enough sterile animals to overload resources, such as food or living space; repetition could lead to complete extinction of the fertile population. This model has since been tested by single releases of sterile animals into populations of the Queensland fruit fly *Dacus tryoni* Frogg (Diptera, Trypetidae).

The most direct method of testing is to release sterile animals into wild populations whose absolute numbers have been estimated, and then to look for sharp decreases in the treated populations in comparison with untreated control populations. Wintering populations of *Dacus tryoni* near Sydney, Australia, were chosen because they had the following characteristics.

1) Apparently discrete, small populations spend the winter in isolated groups of citrus trees. Considerable proportions of such populations may readily be taken for estimates of population by marking, release, and recapture; the variance of such estimates of population is thereby minimized.

2) Earlier observations had suggested that such populations were stable because the birth rate was zero in winter, the death rate was low, and movement was inhibited by isolation and low temperatures. These facts reduced the possibility that accidental fluctuations in numbers might mask the changes effected by introduction of sterile animals.

During the winter (May–August) of 1963 four of seven suitable populations were treated, while three served as controls. Bailey's method of two-point sampling (*2*) was used. Flies were captured with hand nets and marked on the thorax with quick-drying colored paint (*3*); each fly caught received a mark corresponding to the day of capture. Thus was kept a complete record of the dates of capture for all flies caught. After marking, the flies were released into their home trees.

The sterile flies were reared at 25°C and sterilized with 6000 rad of gamma-rays (^{60}Co) on the 8th day of pupal life. After emergence they were held for four days, fed for two days on sucrose containing $^{32}P(NaH_2P^*O_4)$ at a concentration of 100 μc per gram of sugar, and then released into the treated populations. On recapture they were detected by means of a portable counter (*4*); recaptured sterile flies, marked with paint and released like wild flies, were distinguishable by their radioactivity.

The size of each wild population was estimated for a few days before and after the sterile flies were introduced. For accuracy the number of released flies was set at about three to five times the estimated number of wild flies, because a higher ratio might have overdiluted the wild population and prevented us from recovering enough wild flies for the later estimates.

Table 1. Populations of colonies before and after release of sterile flies; standard error, in parentheses, follows each number. Numbers and SE estimated by Bailey's two-point sampling method.

Colony	Population (No.)	
	Before release	After release
C_1	108(51.7)	115 (46.8)
C_2	149(42.1)	95 (29.3)
C_3	88(30.1)	233(130.0)
T_1	193(67.4)	15 (6.1)
T_3	113(46.8)	31 (5.6)
T_4	85(16.8)	27 (9.3)

The results are shown in Fig. 1. The three control populations (C_1, C_2, C_3) were used to determine whether the processes of capturing, marking, and releasing would of themselves lead to a decline in numbers, mimicking the decline expected to follow the release of sterile flies. Apparently two of the control populations (C_1, C_3) and all four of the treated populations (T_1, T_2, T_{3a}, T_{3b}) were stable before the introduction of sterile males. (The apparent fluctuations in numbers reflect unavoidable changes in size of samples and hence in sampling error.) Only C_2 appeared to be unstable, declining slowly throughout the period of observation in a way quite unlike the rapid falls seen in T_1, T_{3a}, and T_{3b} only *after* the releases.

In three of the four treated populations there was a sharp fall in numbers less than 48 hours after entry of the sterile flies. In T_2 the wild population did not decline despite the sterile flies, probably because the latter had been irradiated too early in development; the normal yellow spots on the thorax were bleached to white, a phenocopy induced by radiation in the middle stages of adult morphogenesis within the pupa. Laboratory observations had shown that such flies were very short lived and sluggish; in trial T_2 they tended to be easily caught and to remain in the lower foliage; abnormal behavior and appearance probably prevented them from interacting effectively with wild flies. In T_1, T_{3a}, and T_{3b} the released flies seemed normal in color and behavior. Accordingly, T_2 was rejected when the results were considered. Table 1 compares the most reliable estimates of numbers before and after release in the other six trials,

together with the standard errors of these estimates. In all treated populations the number of irradiated flies fell rapidly within 48 hours, from 500 to 600 on release to 50 to 100; this decrease paralleled the rapid decline of the wild populations in T_1, T_{3a}, and T_{3b}.

The earlier report on the theory of population flushing (*1*) predicted that the number of fertile animals remaining after a single release of sterile animals would be $N_o^2/(N_o + N_s)$, where N_o was the original number of fertile animals and N_s was the number of sterile animals released. This prediction depended on assumption that the original population was already fully loading an inconsumable resource (for example, space), that each sterile animal was equivalent to a fertile one and that flushing removed only the exact excess in numbers induced by release of sterile animals. I did not test these assumptions individually but calculated the efficiency (E) of flushing from the formula

$$E = (N_o - N_A)/N_o - [N_o^2/(N_o + N_s)]$$
$$= \frac{\text{observed decline in population}}{\text{expected decline in population}}$$

where N_A is the number of fertile animals surviving the first release of sterile animals. Because populations T_1, T_{3a}, and T_{3b} were stable before the releases, it is reasonable to take the mean of the population estimates before release as equal to N_o, and the final population estimate as equal to N_A. The efficiencies in T_1, T_3, and T_4 are 1.38, 0.83, and 0.83, respectively.

These results agree reasonably well with expectation, thus suggesting that a resource was being almost fully used and that such sites can support only small populations in winter. These first flushes reduced the wild populations to such low levels that we were unable to test the model further by releasing a second lot of sterile flies. If the first flush selects those animals that adhere strongly to a particular site, later flushes will be less effective; such a phenomenon could be used to measure the strength of attachment to a site.

The experiment strongly suggests that flushing can be induced in natural populations of *Dacus tryoni* and raises two questions: (i) Which resource was overloaded by the addition of sterile animals? (ii) What is the ecological value to natural populations of the spacing reaction here displayed? One hypothesis is that the insects were forced out by an induced shortage of food—presumably honeydew from scale insects (fam. Coccidae) (*5*). All treated populations were in trees infested (some heavily) by scale insects. Wintering populations normally persist in such trees for 4 to 5 months. Possibly the wild populations were consuming honeydew or other food as fast as it was produced, but, because of the large number of scale insects and because the flies responded rapidly (2 days is a maximum value set by the limitations of daily sampling), they probably had no time even to search for all the available food before responding.

Another and more likely hypothesis is that the flies were forced to leave because they began to meet other members of the same species more frequently than before. Fighting between members of either sex has often been observed in natural populations of *Dacus tryoni*. Such response to increased social interaction could be a rapid means of dispersing overcrowded populations. Two advantages might follow: (i) local exhaustion of food by overpopulation would be avoided, and (ii) the local population would be scattered to seek new sites. This may be important for the survival of a species whose food as larvae consists of transient crops. For example, the flies that overwinter come from hosts (to larvae) that may be widely separate from suitable wintering sites; and the sites are not necessarily close to the larval hosts in which

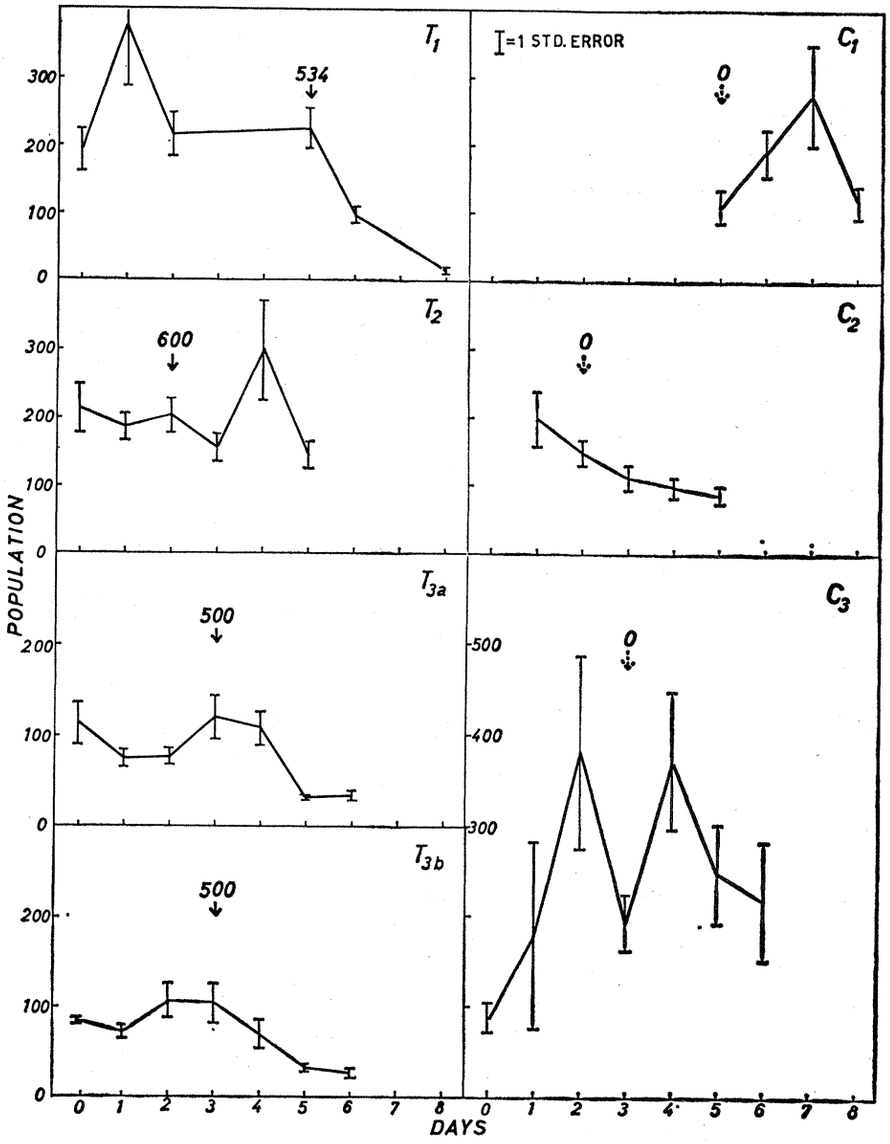

Fig. 1. Population trends in treated (T_1, T_2, T_{3a}, T_{3b}) and untreated colonies (C_1, C_2, C_3). Arrows indicate the introduction of sterile flies. Time scales are comparable within these pairs: T_1-C_1, T_2-C_2, $T_{3a/b}$-C_3.

eggs will be laid in the following spring. A spacing reaction may tend to disperse widely the wintering populations and to increase the proportion of larval hosts that they discover in the following spring.

Another field trial at Warren, New South Wales, suggested that two massive releases of sterile flies at one point in the center of town at the beginning of winter (May 1963) may have driven the wild population to the periphery of town and thus changed the pattern of infestation of the loquat crop in the following spring (September-October) (*6*).

Nadel and Peleg (*7*) have reported a different but related response in the Mediterranean fruit fly, *Ceratitis capitata* Wied. They found that females were more strongly attracted to traps baited with "tri-med" lure (a methylcyclohexane derivative, *7*) when food was short than when food was abundant. Food shortage might be accentuated by release of sterile flies, which might even be used to increase the number of wild flies entering traps or settling on baits.

The efficacy of population flushing is yet to be established, but the procedure is worth developing, both as an ecologic tool and as a means of controlling harmful populations.

J. MONRO*
Waite Agricultural Research Institute, University of Adelaide, South Australia

References and Notes

1. J. Monro, *Science* **140**, 496 (1963).
2. N. T. J. Bailey, *J. Animal Ecol.* **21**, 120 (1952).
3. Dulux or Humbrol.
4. Philips PW-4014, equipped with a thin-window Geiger tube (type 18504).
5. K. S. Hagen, *Proc. Intern. Congr. Entomol. 10th Montreal 1956* **3**, 25 (1958).
6. H. G. Andrewartha, J. Monro, N. L. Richardson, *Australian J. Zool.*, in preparation.
7. D. J. Nadel and B. A. Peleg, *Israel J. Agr. Res.* **15**, 83 (1965).
8. Supported by a research grant from the Waite Institute, University of Adelaide; carried out under the hospitality of L. C. Birch, School of Biological Sciences, University of Sydney. A. Osborn, Waite Agricultural Research Institute, University of Adelaide, gave valuable technical help. The Australian Atomic Energy Commission donated ^{32}P. H. G. Andrewartha and T. O. Browning (University of Adelaide), M. A. Bateman (CSIRO), G. Caughley (New Zealand Forest Service), and D. Nadel (Citrus Board of Israel) read and criticized the manuscript.
* Present address: Joint FAO/IAEA Div. of Atomic Energy in Agriculture, International Atomic Energy Agency, Vienna.

24 January 1966

Marking Single Neurons by Staining with Intracellular Recording Microelectrodes

Abstract. *Glass microelectrodes filled with a saturated solution of methyl blue or fast green in 1.0M potassium acetate can be used to mark penetrated neurons from which intracellular recordings have been made. Many cells can be marked in one experiment and can easily be located in subsequent histological sections.*

When making electrophysiological recordings from the mammalian central nervous system, it is desirable to know the position of the electrode tip and, hence, the site of recorded activity. A number of techniques (*1, 2*) are available for use with extracellular recording electrodes, but these do not permit the exact identification of the neuron from which recordings were made. However, no simple or reliable methods are available for use with intracellular recording electrodes, which allow the study of single nerve cells in effective electrophysiological isolation. In particular, there is no method for marking a series of neurons over a period of hours in one experiment. We have now developed such a method for routinely marking neurons penetrated by microelectrodes, so that the marked cells may be identified easily in subsequent histological sections.

We have described a method for making extracellular marks by passing current through glass electrodes filled with 2*M* sodium chloride saturated with fast green FCF (*2*). It seemed possible that this technique could be adapted for intracellular marking. For this purpose we chose 1.0*M* potassium acetate as the basic solution for the electrodes; this salt does not cause reversal of inhibitory postsynaptic potentials (*3*), and we have found that electrodes filled with it have excellent recording properties.

Saturated solutions of a number of water-soluble dyes in 1*M* potassium acetate were made by shaking an excess of the dye with acetate solution and filtering. Glass microelectrodes, previously filled with distilled water, were individually filled with the dye solutions by means of a fine Pasteur pipette. Alternatively, a number of water-filled electrodes were placed in a large volume of dye solution. The electrodes were then left overnight so that the dye could diffuse to the tips and, in initial trials, were tested to see whether appreciable quantities of the dye could be ejected electrophoretically into agar. Electrodes filled with dyes passing this test were selected for resistances of 5 to 15 megohms and used to penetrate antidromically activated motoneurons in the lumbosacral cord of decapitate cats. When a stable penetration was obtained we attempted to stain the cell by passing a current of a few microamperes through the electrode. The electrode was then withdrawn from the track, so that it did not further penetrate the marked cell. After several test marks were made, segments of the cord containing marks were fixed overnight in 10 percent formalin. In some cases the animal was perfused with saline and formol-saline to remove blood from the cord; this procedure did not seem to affect the marks in any way. Segments of the cord containing test marks were sectioned at 100 μ on a freezing microtome, and the sections were mounted serially on glass slides coated with albumin. The unstained sections were then examined under a dissecting microscope to locate the marked cells. Sections containing marked cells were dried at 50°C for 1 hour before they were stained in thionin and mounted in DPX in the usual manner. In some cases sections were mounted in DPX without staining so that the marked cell could be examined in isolation.

Of the 16 dyes tested, only two have reliably made easily detectable marks—fast green FCF (color index No. 42053) and methyl blue (color index No. 42780), with the latter giving the better results. In the case of both dyes, current was passed with the electrode negative. Electrodes containing these dyes in 1.0*M* potassium acetate have proved excellent for intracellular recording, and the presence of the dye did not affect the impaled cell in any obvious way. Cells impaled for periods of over 30 minutes did not show any deterioration, and one electrode could be used to make several marks in the same experiment.

The appearance of the marked cell varied with the size of the current and the time that elapsed before the animal was killed. The cell shown in Fig. 1 was marked with a methyl blue electrode 9 hours before the animal was killed. The color in the unstained