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# Effect of 6-MBOA on Microtus pinetorum and Microtus pennsylvanicus of different ages

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Short-lived small rodents require environmental cues to synchronize their breeding with optimal environmental conditions. In the absence of such cues animals would waste a large portion of their reproductive effort when environmental conditions were unfavorable. Rowan (1938) noted that endocrine systems regulating mammalian reproduction responded to the environmental photoperiodic cues. Bodenheimer (1946) observed that major outbreaks of voles in Palestine could not be correlated with environmental factors such as climate, photoperiod, volume of food resources, or population density, acting either alone or in combination. From those studies he suggested that vole outbreaks were associated with an unknown factor or factors in the food supply. Research on Rocky Mountain species of microtine rodents have demonstrated that they respond to both inhibitory and stimulatory plant compounds which result in their reproduction only during environmentally favorable periods (Negus et al., 1977). These compounds and their effects have been characterized principally in female montane voles (*Microtus montanus*). Berger et al. (1977) reported on the effect of paracoumaric acid (PCA) and ferulic acid (FA), which were isolated from senescent grass, on montane voles and described decreased uterine weight, cessation of follicular development and a cessation of breeding in these voles. Further the action of green vegetation on reproduction was characterized as increased uterine weight, increased follicular development, increased litter size and early attainment of sexual maturity in young voles (Pinter and Negus, 1965; Negus and Pinter, 1966; Pinter, 1968; Negus and Berger, 1971; Berger and Negus, 1974; Negus and Berger, 1977). Sanders et al. (1981) characterized, isolated and identified the active compound from grass as 6-MBOA which is a non-estrogenic compound naturally occurring in most vegetation as one responsible for the reproductive effects reported by others. He demonstrated a dose response for the compound by assaying the uterine weight response of subadult montane voles and determined that maximal uterine responses occur at concentrations the animal normally encounters as a result of eating fresh grass. He additionally reported that ovariectomized voles do not exhibit a uterine weight response and that in laboratory mice increased ovarian weights occur as a result of 6-MBOA stimulation. Berger et al. (1981) reported that in naturally occurring non-reproductive wild populations of montane voles given oat seeds treated with 6-MBOA respond with increased reproductive organ weights and begin to produce litters while oat-supplemented populations did not respond. Both of their methods (intraperitioneal injected or food coated) yielded similar results in response to 6-MBOA. This report will characterize the effect of 6-MBOA on both meadow voles (*Microtus* *pennsylvanicus*) and pine voles (*Microtus pinetorum*). *Intraperitoneal* injection of *voles at two developmental* ages were carried out to determine if 6-MBOA's effects varied with animal age.

## Materials and Methods

All test animals were derived from the existing laboratory colonies maintained on a LD 16:8 photoperiod at 20°C with Wayne Lab blox and water available ad lib. Animals were weaned at 21 days of age and there after maintained in single cages of 3 animals each under the same conditions as the laboratory colonies. At 30 or 45 days of age the injection series was began with animals randomly assigned to treatment type. The minimum sample size for each species was 10 in each age, sex, and type (experimental or control). Experimentals were injected daily for three days with 5 ug of 6-MBOA in .5 cc of solvent. Controls were similiarly handled and injected with .5 cc of solvent. On the fourth day controls and experimentals were sacraficed and data on body weight, adrenal weight, testicular and seminal vessicle weight for males and for females ovarian and uterine weight. Student T tests and analysis of varience procedures were employed in data analysis.

## Results

The results are summarized in Table 1 and clearly indicate that at normal physiological dose rates both species respond at the reproductive organ level to 6-MBOA stimulation. The magnitudes of the response showed an age dependent effect with 45 day old animals of both species and sexes responding significantly. As body weights are not different with respect to treatment and no significant differences occurred in food intake the acceleration of sexual maturation resulted from the 6-MBOA treatment. No significant differences occured in adrenal weights clearly indicating that the animals were not stressed by these treatments.

## Discussion

The use of plants as a cue for reproductive effort is not unique to microtines. Reynolds and Turkowski (1972) found a close correlation between timing of the breeding cycle and winter rainfall in round tailed ground squirrels. Litter sizes were also correlated with winter rainfall. Germination of desert annuals in response to rainfall appeared to be the cue for reproductive effort in round tailed ground squirrels. Similarly, Beatley (1969) and Van de Graaf and Balda (1973) found high correlations between rainfall, germination of annuals, and reproductive effort in heteromyid rodents. Australian red

Table 1. Response of voles on LD 16:8 to intraperiotoneal injection of 6-MBOA at two different ages. A. Microtus pennsylvanicus males. B. Microtus pinetorum males. C. Microtus pennsylvanicus males. D. Microtus pinetorum females. C = controls, E = experimentals. Body weight in grams other weights in milligrams. * Significantly different .05 to .01, ** Significantly different .01 to .001.

| AGE | TYPE | BODY | WEIGHT | ADRENALS | SEMINAL VESSICLES | TESTES |
|---|---|---|---|---|---|---|
| A | 30 days | C | 25.9 ± 2.0 | 122 ± 27 | 129 ± 128 | 3387 ± 1816 |
| | | E | 26.4 ± 2.0 | 141 ± 29 | 89 ± 47 | 3414 ± 1578 |
| | 45 days | C | 31.4 ± 0.9 | 127 ± 8 | 215 ± 48 | 2739 ± 652 |
| | | E | 33.1 ± 0.8 | 126 ± 7 | 602 ± 126** | 6326 ± 806** |
| B | 30 days | C | 20.8 ± 3.4 | 91 ± 24 | 141 ± 97 | 411 ± 136 |
| | | E | 20.2 ± 2.8 | 92 ± 23 | 200 ± 98* | 464 ± 185 |
| | 45 days | C | 22.6 ± 3.0 | 115 ± 19 | 276 ± 125 | 546 ± 196 |
| | | E | 21.6 ± 3.0 | 122 ± 17 | 490 ± 199** | 1079 ± 565** |
| AGE | TYPE | BODY | WEIGHT | ADRENALS | OVARIAN | UTERINE |
| C | 30 days | C | 25.5 ± 5.0 | 150 ± 35 | 52 ± 18 | 124 ± 36 |
| | | E | 27.8 ± 2.0 | 157 ± 38 | 64 ± 23 | 175 ± 53* |
| | 45 days | C | 26.1 ± 2.0 | 147 ± 38 | 61 ± 20 | 145 ± 34 |
| | | E | 27.0 ± 2.0 | 157 ± 43 | 82 ± 33* | 424 ± 34** |
| D | 30 days | C | 19.8 ± 2.4 | 82 ± 16 | 46 ± 18 | 90 ± 32 |
| | | E | 21.7 ± 2.2 | 87 ± 18 | 50 ± 16 | 109 ± 56 |
| | 45 days | C | 22.8 ± 2.7 | 104 ± 22 | 59 ± 18 | 121 ± 37 |
| | | E | 22.7 ± 3.1 | 113 ± 16 | 78 ± 20** | 196 ± 70** |

kangaroos (Megalea rufa) will enter estrus and breed within a few days after a rainfall and flush of new grass growth (Newsome, 1964). European rabbits (Oryctolagus cuniculus) in Australia exhibit similar responses to rainfall and grass growth (Poole, 1960). In all of these examples, herbivores depended on the vegetative condition of plant materials and have synchronized reproduction with its seasonal availability. Within these species, reproduction is maximized as a result of the response to plant chemical signals which would be predictive of the onset of good environmental conditions regardless of the other cues, particularly photoperiod.

Our recent work with Microtus pennsylvanicus, a close relative of Microtus montanus, indicates that this species responds to photoperiodic information similar to that response reported by Petterborg (1978) for M. montanus with respect to changes in body weight and growth rates as a function of photoperiod (Pistole and Cranford, 1982). Additionally, both PCA and FA (reproductive inhibitors) have been shown to depress uterine weight in M. pennsylvanicus and not in Peromyscus maniculatus (Cranford et al., 1980). The uterine depression in M. montanus occurred at physiological doses (Berger et al., 1977) but in M. pennsylvanicus twice the physiological dose (pharmacological dose) was required to produce the same response. These similarities and differences related to the differences in habitat between western voles (unpredictable habitats) and eastern voles (predictable habitats) voles and the evolutionary selection of differential responses to these environmental cues. Preliminary results from small scale studies with 6-MBOA demonstrate that M. pennsylvanicus and M. pinetorum respond to the same dosage rates reported by Berger et al. (1981). These studies indicate that both males and females respond to the stimulatory compound and that this is a more generalized biological response. These studies clearly point to a need for further studies to evaluate 6-MBOA on a selected set of species at different ages and under different photoperiodic conditions. These data additionally indicate that the maintenance of a vegetative understory, particularly grasses, in an orchard accelerates the rate of sexual maturation. This could result in higher rates of reproduction within an orchard due to sexual maturation being reached 4 to 6 weeks earlier than a non-stimulated population.

## Literature Cited


Beatley, J. C. 1969. Dependence of desert rodents on winter annuals and precipitation. Ecology, 50:721-724.

Berger, P. J., and N. C. Negus. 1974. Influence of dietary supplements of fresh lettuce on ovariectomized *Microtus montanus*. J. Mamm., 55:747-750.

Berger, P. J., E. H. Snaders, P. D. Gardner, and N. C. Negus. 1977. Phenolic plant compounds functioning as reproductive inhibitors in *Microtus montanus*. Science, 195:575-577.

Berger, P. J., N. C. Negus, E. H. Sanders, and P. D. Gardner. 1981. Chemical triggering of reproduction in *Microtus montanus*. Science, 214:69-70.

Bodenheimer, F. S. 1946. The estrous cycle of *Microtus guentheri* D. and A. and its ecological *implications*. *Ecology*, 27:255-256.

Cranford, J. A., D. H. Pistole and T. L. Derting. 1980. Results of chemical inhibitors and photoperiodic influences on growth and reproduction in *Microtus pennsylvanicus*. Proceedings 4th Pine Vole *Conference*. *Ed. by R. E*. Byers. Hendersonville, NC. pp 28-33.

Iverson, S. L., and B. N. Turner. 1974. Winter weight dynamics in *Microtus pennsylvanicus*. Ecology, 55:1030-1041.

Negus, N. C., and P. J. Berger. 1971. Pineal weight response to a dietary variable in *Microtus montanus*. Experientia, 27:215-216.

Negus, N. C., and P. J. Berger. 1977. Experimental triggering of reproduction in a natural population of *Microtus montanus*. Science, 196:1230-1232.

Negus, N. C., and A. J. Pinter. 1966. Reproductive responses of *Microtus montanus* to plants and plant extracts in the diet. *J. Mamm., 47:596*-601.

Negus, N. C., P. J. Berger, and L. G. Forslund. 1977. Reproductive strategy of *Microtus montanus*. J. Mamm., 58:347-353.

Newsome, A. E. 1966. The influence of food on breeding in the red kangaroo in central Australia. CSIRO Wildlife Res., 11:187-196.

Petterborg, L. J. 1978. Effect of photoperiod on body weight in the vole Microtus montanus. Canadian J. zool., 56:431-435.

Pinter, A. J. 1968. Effects of diet and light on growth, maturation and adrenal size of Microtus montanus. Amer. J. Physiol., 215:461-466.

Pinter, A. J. and N. C. Negus. 1965. Effects of nutrition and photoperiod on reproductive physiology of Microtus montanus. Amer. J. Physiol., 208:633-638.

Pistole, D. H., and J. A. Cranford. 1982. Photoperiod effects on growth in Microtus pennsylvanicus. J. Mamm., 63:547-553.

Poole, W. E. 1960. Breeding in the wild rabbit, Oryctolagus cuniculus (L) in relation to the environment. CSIRO Wildlife Res., 5:21-43.

Reynolds, H. G., and F. Turkowski. 1972. Reproductive variations in the round-tailed ground squirrel as related to winter rainfall. J. Mamm., 53:893-898.

Rowan, W. 1938. Light and seasonal reproduction in animals. Biol. Rev., 13:374-401.

Sanders, E. H., P. D. Gardner, P. J. Berger, and N. C. Negus. 1981. 6-Methoxybenzoxazolinone: A plant derivative that stimulated reproduction in Microtus montanus. Science, 214:67-69.

Van de Graaf, K. M., and R. P. Balda. 1973. Importance of green vegetation for reproduction in the kangaroo rat, Dipodomys merriami merriami. J. Mamm., 54:509-512.

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