roid, and mammary gland (*2*, *16*). Our report could serve as a model for the use of hydroxyurea in experimental carcinogenesis.

PO CHUEN CHAN*
ALLAN GOLDMAN
ERNEST L. WYNDER*
Sloan-Kettering Institute for Cancer Research, New York 10021

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17. Supported by grants from the American Cancer Society (E-231), Spenadel Fund (No. 459), and in part by NCI grant CA 08748. We thank Dr. F. S. Philips for suggestions and Dr. D. Hoffmann for supplying purified DMBA.
* Present address: American Health Foundation, 120 East End Avenue, New York 10028.
21 April 1969; revised 19 January 1970

Dodder Weevils in Simultaneous Association with Parasitic Plants and Their Hosts

Abstract. *The weevil* Smicronyx quadrifer (*broad sense*) *is restricted in host preference to the parasitic dodders* (Cuscuta *species*) *during its adult life, but its larvae consistently move from dodder stems into living stems of certain hosts of dodders, where they feed, grow to maturity, and undergo metamorphosis. Such associations, involving three interacting organisms, are very unusual among phytophagous insects.*

Dodders (*Cuscuta* spp.) are vinelike, yellow or orange parasitic plants that grow attached to a wide variety of other plants in many parts of the world (*1*). In turn, they have some permanent insect associates (or parasites), including aphids (*2*), a small fly (*3*), a moth (*3*), and weevils of the genus *Smicronyx* (*4*). The weevils have been found developing in dodder stem galls and seed capsules in North and South America, Europe, Asia, and North Africa (*4*), and a few Old World species are associated with parasitic plants of the genera *Orobanche* (*5*) and *Striga* (*6*). In North America, larvae of several species of *Smicronyx* feed in the seeds of certain Compositae, such as *Ambrosia* spp. and *Helianthus* spp., and a few unusual records indicate that stems of Compositae have been attacked, when infested with dodder, by larvae of *Smicronyx sculpticollis* Casey, a species associated with dodder (*4*). I now report a very unusual host relationship in which *Smicronyx quadrifer* Casey (broad sense) (*7*) is consistently associated with dodder (*Cuscuta gronovii*, *C.* sp., probably *pentagona*) (*8*) and certain hosts of the dodder. This unusual insect-plant association involves three, rather than the usual two, interacting organisms, and it may be significant to other studies involving dodders, their hosts, and other organisms (see *2*, *9*).

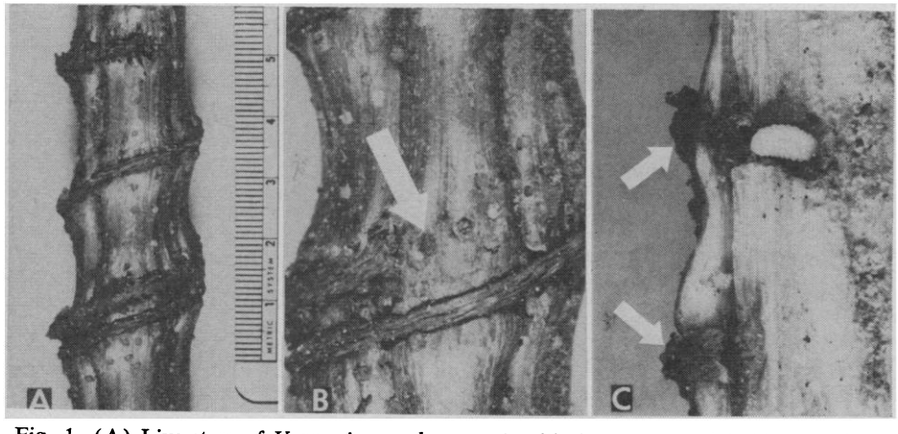

Fig. 1. (A) Live stem of *Vernonia noveboracensis* with dry remains of dodder attached; scale is marked in centimeters. (B) Enlarged view of the stem with part of the dodder removed, showing position of larval entry hole (now filled with excrement), made through dodder attachment (arrow). (C) Longitudinal section of stem, showing partly grown *Smicronyx* larva burrowing inward from tunnel under dodder (arrows indicate dodder).

During the summer of 1968, I investigated the dodder-infested stems (Fig. 1A) of seven perennial species of Compositae, identified as *Vernonia noveboracensis, Eupatorium rugosum, E. perfoliatum, E. fistulosum, Aster puniceus, Artemisia vulgaris,* and *Solidago* sp. (*8*), growing near the Chesapeake and Ohio Canal in Washington, D.C. and Maryland. Many of these stems contained weevil larvae identifiable as *Smicronyx* sp., burrowing in and near the areas in which the dodder was attached. The pattern of excrement-filled larval tunnels indicated that the larvae had mined out short sections of dodder stem, then burrowed through the sucker-like haustoria into the stems of the dodder hosts (Fig. 1B). They usually had tunneled just beneath the dodder before they chewed their way inward (Fig. 1C) and formed pupal cells in the cortex or in the pith of the hosts. Dry stems remaining from the previous summer showed the same pattern of tunnels and yielded the remains of a few adult *Smicronyx quadrifer*. Dodder-infested plants representing seven families other than Compositae were growing in the same areas, but I found no *Smicronyx* larvae in their stems.

In the succeeding fall and winter approximately 170 adult *S. quadrifer* and 1 *S. sculpticollis* were treated from 110 stems (15 to 22 cm long) representing all seven plant species in which larvae had been found. Concurrent rearings of larvae from dodder stems and fruit stripped from the composite stems or from plants growing in the same areas produced 72 adults representing five species of *Smicronyx*, including *S. sculpticollis*, but no *S. quadrifer*. Thus, the larvae of the latter species cannot, or normally do not, reach maturity in the tissues of dodder alone, although that

is the usual habit of the other species reared.

The *S. quadrifer* larvae are adjusted to, and dependent upon, the completion of their growth and metamorphosis in the stems of dodder hosts that they invade. Dissection of stems bearing dodder showed that after hatching in the dodder stem in June or July the young larvae had consumed most of the tissues in short sections of it, then penetrated the stem of the dodder host where they burrowed and fed until they had grown to their mature size by October (*10*). After chewing exit holes from their pupal cells to the surface and plugging the holes with excrement and detritus, they overwintered in the stems and pupated there in late April (in the field).

The adults are primarily associated with dodder. In the laboratory, they were strongly attracted to mature plants, seedlings, open seeds, or seed juices absorbed in paper, and they fed on the tender stem tips and buds. However, their egg-laying habits associate them with the dodder hosts that the larvae invade. Several females confined with males on dodder-infested *Solidago* plants in the laboratory deposited their eggs only in those portions of dodder stem that were directly attached to the stems of the hosts. When confined on loosely hanging portions of the dodder, the same females did not oviposit at all. In the field, mating pairs of *Smicronyx quadrifer* were found on dodder attached to *Vernonia noveboracensis*, *Artemisia vulgaris*, and *Solidago* sp., and the pattern of egg deposition associated with them was the same as that observed in the laboratory.

Female *Smicronyx sculpticollis* confined with males on dodder-infested *Solidago* plants laid very few eggs in the dodder stems. Only one living larva was recovered from the dodder stems, and no larval penetration could be detected in the *Solidago* stems. The fact that a few eggs were deposited in the dodder stems might, however, account for the occasional rearing of *Smicronyx sculpticollis* from hosts of dodder.

The factors underlying the development and maintenance of the host preferences shown by *S. quadrifer* will require further clarification. However, one apparent factor is the site of oviposition. Larvae hatching from eggs placed in the dodder stem near the haustoria are not only in position to enter the stem of the host of the dodder but may have to do so or perish, because the diameter of most dodder stems (0.8 to 2.0 mm in my material) is not large enough to accommodate mature larvae, which average 1.3 mm in body width, particularly after the dodder dies and shrinks with desiccation.

If the previously suggested evolution of host preferences (that is, from dodders to Compositae) in North American species of *Smicronyx* (*4*) is correct, the association described here may be significant as an intermediate stage in that sequence. There are apparently few recorded instances of a shift from one preferred host to another that are supported by well-documented intermediate stages (*11*).

D. M. ANDERSON*
Systematic Entomology Laboratory, Agricultural Research Service, Washington, D.C.

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7. The name *Smicronyx quadrifer* is applied in the broad sense here, because of my uncertainty of the taxonomic status of the eastern and western populations of the species.
8. The *Cuscuta* species were identified by Dr. E. E. Gaertner, Chalk River, Ontario; all other plant identifications were reviewed by Mr. C. V. Morton, Department of Botany, U.S. National Museum of Natural History.
9. R. M. Gilmer [*Phytopathol.* **48**, 432 (1958)] and other authors have described their use of dodder as a living bridge to transmit viruses from plant to plant.
10. Growth of larvae in stems of hosts of dodder can be demonstrated by measurement. Head widths of 34 young larvae dissected from dodder-infested stems of *Artemisia vulgaris* on 8 August 1969 varied from 0.20 to 0.42 mm, averaging 0.34 mm, whereas those of 47 mature larvae collected from the same situation on 18 October 1969 varied from 0.43 to 0.51 mm, averaging 0.47 mm.
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* Mailing address: c/o U.S. National Museum of Natural History, Washington, D.C. 20560.

8 December 1969

Mixed Lymphocyte Cultures Produce Effector Cells: Model in vitro for Allograft Rejection

Abstract. *Mouse peripheral lymphocytes sensitized in vitro by culturing with allogeneic lymphocytes produced immunospecific destruction of target cells, as measured by release of chromium-51. Thus the sensitizing and effector phases of the cell-bound immune response can both be studied in an in vitro system.*

Immunity to allografts has been studied in vitro with the use of four independent test systems. The first is based on the proliferative response of nonimmune lymphoid cells when cultivated with allogeneic cells (the mixed lymphocyte interaction, MLI) (*1–3*) or with a cell-free preparation of the transplantation antigen (or antigens) (*4*). A second system is based on the destruction of target cells by lymphoid cells obtained from already immunized donors (*5, 6*). In a third type of test (*7*), target cells are cultivated with nonimmune allogeneic lymphocytes in the presence of phytohemagglutinin (PHA); this also results in the destruction of the target cells, presumably through a stage of lymphocyte blastoid transformation and proliferation initiated by the mitogen. In the fourth kind of test, the motility of macrophages is blocked by a combined action of antigen, sensitized lymphoid cell, and a factor released to the medium (*8*).

The immunologic specificity and selectivity of the proliferative phase of the MLI have been demonstrated (*2, 9*). It has been assumed that the reaction is directed against the (dissimilar) histocompatibility antigens of the stimulating lymphocyte population (*2, 3*) and thus represents in vitro the initial or "sensitizing" phase of the allograft immune response. If the production of effector cells could be demonstrated in these allogeneic lymphocyte cultures, this would give the final proof of the true immunologic nature of the MLI and make possible the study of allograft immune response in a genetically defined system in vitro. We tested this possibility, using mixed cultures of mouse peripheral leukocytes. In this test, the sensitizing phase of the immune response is characterized by incorporation of radioactive precursors into the replicating DNA of the responding lymphocyte population and the destructive phase by release of ^{51}Cr from labeled target cells undergoing cytolysis.

Under conditions already described