

The Harefoot Mushroom, *Coprinus lagopus* Fr., on Fruits Used Commercially as Seedstocks¹

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Sporophores of the black-spored mushroom, *Coprinus lagopus* Fr., have developed saprophytically from fruits of several plants during germination testing of the enclosed seeds. They have been observed frequently on fruits of European-grown New Zealand spinach since 1940 and occasionally on seedballs (botanical fruits) of garden beets. The pileus of a sporophore was small in proportion to the stipe, 4 to 28 mm across, tender, at first cylindrical, covered with fugacious fibrils, at maturity becoming campanulate, then flattened, smooth, with edges revolute or split. A mature, fully elongated stipe was typically long, slender, hollow, fragile, and bulbous, 5 to 26 cm high, 2 to 7 mm in diameter, white and wooly. The gills were white when young, then black, free, remote, linear, and very thin. The spores were black, elliptical, easily visible through the thin flesh of the pileus, and averaged 11.7 to 13.1×6.3 to 7.4 μ .

The sporophores occurred singly, scattered, or in crowded clumps, of 20 to 30 on a fruit, seedball, or other nonliving structure enclosing the true seeds. The first sporophores of *Coprinus lagopus* usually matured in about 24 days after the infested fruits had been placed in a germinator operated at an alternating temperature of 20° and 30° C. During ripening, the stipes of the mushroom elongated rapidly, the pilei became flattened, and the gills deliquesced completely within a few hours. When removed from a humid germinator to the more arid laboratory, young sporophores arising from any medium invariably withered and failed to mature.

As previously suggested (5), the mushroom was recognized as *Coprinus lagopus* Fr. According to Saccardo (7), the spore sizes of this species are 12 to 13×6 to 8 μ , while the pileus is tender, 2 to 3 cm long, and the stipe is fragile. Rea's (6) description of *C. lagopus* is applicable, except in spore measurements of 10 to 12×6 to 7 μ , to the specimens observed at Geneva. Kaufman (3), in classifying 30 species of *Coprinus* of Michigan, included no specific description of *C. lagopus* but did state that *C. tomentosus* Fr. may be the *C. lagopus* of various authors. The mushrooms observed on fruits at Geneva, and at State College, New Mexico clearly did not conform to Kaufman's (3) description of *C. tomentosus*, although the spore sizes were parallel.

Previously Rea (6) and Pape (4) have identified agarics found on seedballs of beets and mangels as *Coprinus pilosus* Beck. and *C. nycthemerus* Fr. respectively.

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A few specimens growing on fruits of New Zealand spinach and seedballs of beets at Geneva might have been recognized as one of these species had not continued culturing produced the typically long-stemmed fruit bodies of *C. lagopus*. It is probable that both Rea and Pape would have assigned the beet-infesting mushroom to *C. lagopus* had they transferred their cultures to richer media.

Successful transferring of both the beet and New Zealand spinach strains to manure, rotten wood, and straw has shown that the size of the sporophore is somewhat dependent upon the medium. The transfers also furnished proof that the strains isolated at Geneva readily grow on the same media, manure and rotten wood, mentioned by Rea (6) and Pape (4) for English and German strains.

Since the first observation of *Coprinus lagopus* upon European-grown fruits of New Zealand spinach, the mushroom has fruited on garden beet seedballs, on the berry-like fruits or arils of yew (*Taxus cuspidata capitata* Sieb & Zacc.), on the calyx and bracts surrounding seeds of pincushion flower (*Scabiosa caucasica* Bieb.), and on glumes enclosing caryopses of bluestem wheatgrass (*Agropyron smithii* Rydb.), and of crested wheatgrass (*A. cristatum* Gaertn.). The sporophores of a *Coprinus* were previously (1) reported from the fruit or locumtum of sainfoin, *Onobrychis viciaefolia* Scop.

Fruit bodies of the larger species of *Coprinus* commonly collected for food have never developed from any commercial seedstock tested at Geneva. Attempts to establish these species in fruits of New Zealand spinach and in seedballs of garden beets have been entirely unsuccessful.

Apparently the mycelium of *Coprinus lagopus* was borne within the fruits, seedballs, glumes, or similar plant parts. Fungus hyphae have been demonstrated in those tissues from which sporophores developed. Furthermore, surface sterilization of New Zealand spinach fruits and of beet seedballs did not inhibit development of the sporophores of *C. lagopus*. The hyphae have not been demonstrated in the botanical seeds enclosed in fungus-infested fruits, and sporophores have never arisen from the seeds of any species.

Spores of *Coprinus lagopus* have not been found on seeds, fruits, or other plant structures received for routine germination testing. In fact, no evidence has been found to indicate that spores do, or even could, perpetuate the fungus.

The medium, whether a seed-enclosing structure or disorganized tissue, visibly affects the size of the fruit bodies. The stipes arising from glumes enclosing seeds of *Agropyron* spp. both at State College, New Mexico, and at Geneva were only 2 to 5 cm high, with pilei of 7 to 12 mm diameter. With successive transfers to blotting paper, New Zealand spinach fruits, and beet seedballs, the fruit bodies progressively increased in size. The larger stipes from the latter medium averaged 14 cm in length while the ripened pilei averaged 26 mm in diameter.

The size of the sporophore had only slight influence or none upon the size of the spores. The averages of 12 to $13 \mu \times 6$ to 8μ as specified by Saccardo (7) were

applicable to spores dropping from 95% of the sporophores. The kind and size of fruit or similar plant part, while affecting the size of the sporophores, did not exert a similar influence upon the spores. The size of the spores remained very constant when a strain of *Coprinus lagopus* from *Agropyron smithii* was successively cultured on glumes of *A. cristatum*, seedballs of beets, and fruits of New Zealand spinach.

The size of the sporophores, the constancy of spore measurements, the ease of transfer of the fungus from fruits of one plant species to those of others, and the failure of the large edible species to grow upon fruits indicate that the harefoot mushroom is the species of *Coprinus* that has been observed at Geneva, New York, and at State College, New Mexico.

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The Validity of the Use of Tracers to Follow Chemical Reactions

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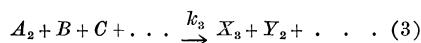
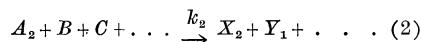
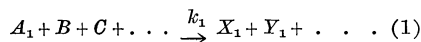
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In the use of tracers, either stable or radioactive, it is important to know how much difference in chemical properties there is between the isotopic molecules. The thermodynamic properties of systems containing isotopic molecules in chemical equilibrium can be calculated by simple formulae (1).

Recently Urey (4) tabulated the equilibrium constants of various exchange reactions for isotopic compounds of elements in the first row of the periodic table, as well as the halogens. In many cases the isotopic molecules are not in equilibrium with respect to various exchange reactions. In such cases it is important to know whether or not the specific activity of the product of some chemical transformation of the substrate isotopic molecules is the same as the initial specific activity. If the initial specific activity is known, then the specific activity of the product can be calculated by integration of the rate equations for the respective reactions. In this paper we shall neglect changes in the specific activity by radioactive decay or other nuclear processes. These corrections can be made by the use of the equations of radioactive transformation (3).

We shall consider the general case where the isotopic molecules A_1 and A_2 react either with each other or with other molecular species B , C , etc. to give the isotopic molecules X_1 and X_2 amongst the products. Frequently the isotopic molecule A_2 may be a mixed molecule and

contain two different isotopes of one element in chemically equivalent positions, e.g., CH_3D . A mixed molecule may react in one of two ways: It may give the products X_2 , Y , etc. or X_3 , Y_2 , etc. In most cases X_3 will be the same as X_1 but we shall retain the symbol X_3 to avoid confusion. If the isotopic molecule A_2 is present only in tracer amounts, then the reaction will always be first order in A_2 . If a_1^0 and a_2^0 are the initial concentrations of A_1 and A_2 respectively, then the amounts of X_1 , X_2 and X_3 , dx_1 , dx_2 and dx_3 respectively, formed in a time interval dt at the time t by reaction according to the following equations



are

$$dX_1 = k_1(a_1^0 - lX_1)^n(B)^b(C)^c \dots dt \quad (4)$$

$$dX_2 = k_2(a_2^0 - lX_1)^{n-1}(B)^b(C)^c \dots dt \quad (5)$$

$$dX_3 = k_3(a_2^0 - lX_1)^{n-1}(B)^b(C)^c \dots dt \quad (6)$$

where

$$a_2 = a_2^0 - mX_2 - nX_3.$$

The coefficients l , m , and n are small numbers which are derived from the stoichiometry of the reaction.

From equations (5) and (6) it follows that

$$dX_3 = (k_3/k_2) dX_2 \quad (7)$$

and

$$X_3 = (k_3/k_2) X_2 \quad (8)$$

Thus

$$a_2 = a_2^0 - (m + nk_3/k_2) X_2 \quad (9)$$

From equations (4), (5), and (9) we get

$$\frac{k_2}{k_1} \int_0^{x_1} \frac{dX_1}{(a_1^0 - lX_1)^n} = \int_0^{x_2} \frac{dX_2}{a_2^0 - (m + nk_3/k_2) X_2} \quad (10)$$

Integration of equation (10) gives

$$\frac{X_2}{a_2^0} = \frac{1 - (1 - lX_1/a_1^0)^{k_2(m+nk_3/k_2)/k_1 l}}{m + nk_3/k_2} \quad (11)$$

If we define the specific activity of the product as $N_x = X_2/X_1$ and the initial specific activity as $N_A^0 = a_2^0/a_1^0$, then

$$\frac{N_x}{N_A^0} = \frac{1 - (1 - lX_1/a_1^0)^{k_2(m+nk_3/k_2)/k_1 l}}{(m + nk_3/k_2) X_1/a_1^0} \quad (12)$$

For small amounts of conversion, $lX_1/a_1^0 \ll 1$,

$$\frac{N_x}{N_A^0} = k_2/k_1 \quad (13)$$

At complete conversion, $X_1 = a_1^0/l$,

$$\frac{N_x}{N_A^0} = \frac{l}{m + nk_3/k_2} \quad (14)$$

In a forthcoming publication from this laboratory (2) a general equation is derived for the ratio of the rate constants for reactions involving isotopic molecules. For reactions which occur at room temperature and above, the ratio of the rate constants can be expressed approximately by the equation

$$\frac{k_1 s_1 \ddagger s_2}{k_2 s_2 \ddagger s_1} = \left(\frac{m_2^*}{m_1^*} \right)^{1/2} \left(1 + \frac{3n-6}{\sum_i G(u_i) \Delta u_i} - \frac{3n^1-6}{\sum_i G(u_i \ddagger) \Delta u_i \ddagger} \right) \quad (15)$$