## Sexual reproduction of yellow ecotype of *Neurospora intermedia* in nature

A. Pandit, P. S. Dubey and S. Mall – Institute of Environment Management and Plant Sciences, Vikram University, Ujjain (M.P.) 456 010, India

The vegetative stage of yellow ecotype of *Neurospora intermedia* occurs on various substrates but there has been no evidence of its telomorphic stage in nature. This paper presents evidence for the sexual reproduction of this ecotype in nature on maize cobs.

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The samples of *Neurospora intermedia* collected from burnt vegetation from Maddur (Pandit and Maheshwari 1996 J. Biosci. 21: 57-79) and throughout the world are pinkish or "salmon" orange colored and are referred as the orange ecotype (Perkins and Turner 1988 Exp. Mycol. 12: 91-131). In contrast many *Neurospora intermedia* samples collected from substrates other than burned vegetation such as roasted or cooked maize cobs, filter mud and onchom are golden orange or saffron yellow colored and are commonly referred as the yellow ecotype (Turner 1987 Mycologia 79: 425-432; Shaw 1990 The Mycologist 4: 6-13). The yellow ecotype is never found on burnt vegetation although orange strains are some times present along with the yellow strains on discarded maize cobs (Personal observations; Turner 1987 Mycologia 79: 425-432). The pink strains colonizing burnt sugar cane reproduce sexually in nature (Pandit and Maheshwari 1996 J. Biosci. 21: 57-79), but there has been no evidence for sexual reproduction of yellow ecotype in nature. Moreover, it has been experienced that the yellow strains have poor fertility under standard crossing conditions in the laboratory. Thus it was of interest to explore whether the yellow strains reproduce sexually in nature.

Discarded maize cobs "infected" with yellow ecotype of *Neurospora* can be commonly found on the grounds of the Railway station in Ujjain City during July to October, during monsoon season. The cobs experience intermittent dry and rainy spells during the entire season.

In order to examine if both mating types of *Neurospora* are present on the same cob, 35 well-isolated pustules of *Neurospora* from 16 different infected cobs were collected. All the isolates collected were *Neurospora intermedia* with 18 *A* and 17 *a* mating type. In five of 16 cobs, strains of both mating types were present.

These *Neurospora* infected cobs were incubated in a tray (uncovered) in an open garden area and were regularly examined to study the progress of sexual reproduction. Immature perithecia could be seen on the cobs 7-10 days later. The perithecia were usually found in the groove that had been formed after the removal of the kernel from the cob and were mostly in clusters. At the time of perithecia formation the conidia almost disappeared (but in some cases conidia were present), either they were washed off by the heavy rains or were foraged by the insects and their larvae that were seen foraging on the conidia. About two weeks later some perithecia were dissected, revealing different developmental stages (Fig. 1A). The ascospores measured 25 X 17μm and showed distinct grooves characteristic of *Neurospora* (Fig. 1B). When these ascospores were germinated on Vogel's N media after 60°C heat shock for 30 minutes, they produced yellow conidiating culture of *N. intermedia*. Therefore, the perithecia on cobs were formed by the sexual reproduction of the yellow strains.

In three different samplings during this season perithecia were found on 7 of 29 *Neurospora* infected cobs. Hence, the fertility of the yellow strains is relatively high. The yellow ecotype found on cobs apparently reproduces immediately after the vegetative phase whereas the orange strains found on sugarcane form perithecia 4 – 6 months after the vegetative stage (Pandit and Maheshwari 1996 J. Biosci. 21: 57-79).

The yellow ecotype of *Neurospora intermedia* has poor fertility under standard crossing conditions (Turner 1987 Mycologia 79: 425-432). Towards this the yellow strains collected from cobs in Delhi and Ujjain were crossed with each other in different combinations on media of different composition to investigate the factors that limit their fertility under laboratory conditions. Ten crosses were made using 20 different yellow cultures. Three media were used – 1) Westergaard and Mitchell's Synthetic crossing medium, 2) 2% water agar with 2% maize cob powder and 3) 1cm X 2 cm piece of dried maize cob (approx. 0.5 g) autoclaved in 1ml distilled water. In the two agar media cases the crosses were made by the standard procedure in which the protoperithecial parent was inoculated on the crossing tube and fertilized with conidia after 5-7 days. On the cob piece *A* and *a* strains were inoculated together. The crosses were incubated at 25°C.

Our results confirm that the yellow strains are highly infertile on standard crossing media. However, the same strains are extremely fertile if the cross is made on a small piece of cob. In all the crosses abundant perithecia were formed which produced enormous amounts of black ascospores. The cob powder did not increase fertility on agar medium. To test whether substrate or the method of crossing affected fertility, further crosses were made on synthetic cross medium and cob powder medium by coinoculating *A* and *a* strains. Perithecia production was increased by coinoculation, but fertility was not as high as on cob pieces.

The fertility of *N. sitophila* is dramatically increased by substituting filter paper for sucrose in Westergaard's medium (Fairfield and Turner 1993 Fungal Genet. Newsl.40: 30-31). In the case of yellow *N. intermedia*, however, it may be that both the physical structure of the cob as well as the nutrient source affect the fertility.

The time, place and substrate for the occurrence of both the ecotypes in nature are entirely different. It seems that in both the ecotypes the essential requirement for sexual reproduction is cellulose and xylose rich substrate which is available to yellow ecotype at the inception of the vegetative phase in the form of cobs, but the orange strains which begin their life-history on the juicy sugarcane have to wait to reproduce sexually till the substrate is transformed to a sugar-depleted cellulosic rich substrate. The presence of rains and the microenvironment which is in the form of groove in the cobs and cavity below the epidermis in the case of sugarcane also seem critical, which may provide optimum moisture, temperature and other requirements for crossing.

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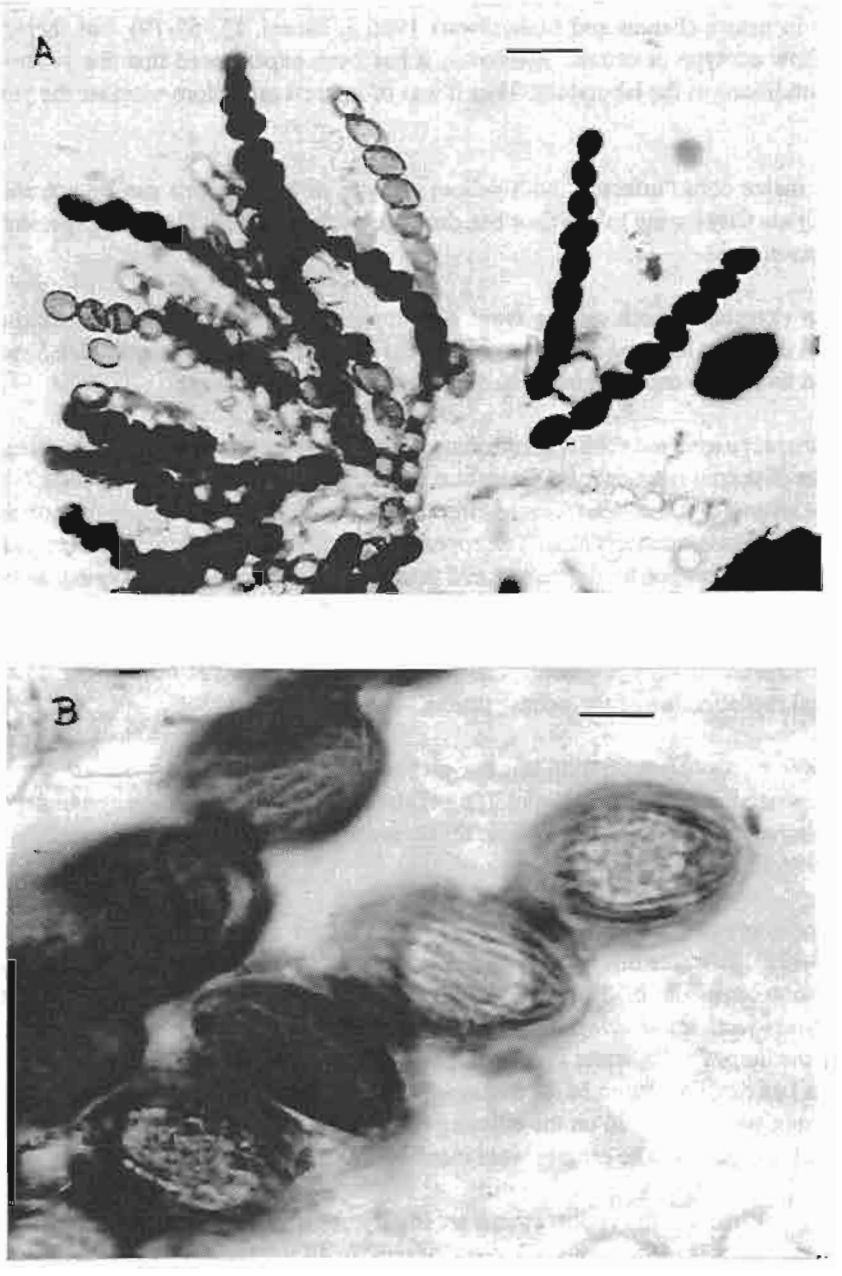


**Fig. 1.** A. Ascospores in different stages of development (Bar, approx. 38 μm). B. Magnified view showing grooves (Bar, approx. 0.8 μm).