Induction of Mitotic Abnormalities in the Root Tips of *Allium cepa* by Tobacco Smoke[1]

Previous studies have revealed that cigarette smoke, its condensate and some fractions have a profound effect on the cytology of cells in vivo or in vitro[2–9]. While working with the root-tips of some higher plants, Izard and his coworkers[7–9] observed that tobacco smoke condensate and some of its components induce mitotic abnormalities in the cells. The aim of the present study was to extend the basic knowledge regarding the effect of cigarette smoke on cell division. Since experimentation with plants is generally easier and quicker than with animals, investigations were conducted to study the effect of cigarette smoke on the cytology of young onion seedlings grown in vitro. An attempt was made to ascertain the feasibility of using plant tissues for rapid bioassay to detect the cytological effects of tobacco smoke.

Seeds of *Allium cepa* (Onion), obtained from Ferry Morse Seed Company, Mountain View, California, USA, were utilized in the present investigation. The seeds were arranged on a sterile moist filter paper in sterile petridishes. After 5 days, when root length was about ¾–1 inch, the seedlings were exposed to different numbers of puffs of tobacco smoke from a smoking machine using a 35 ml puff volume. The petri-dish (without cover) containing germinating seeds was placed in the specimen dish (10 cm diameter, 4 cm height). The dish was then tightly covered with aluminium paper. From one of the sides, the aluminium paper was lifted, the smoke puff introduced and the dish covered. Puffs were given at 10 min intervals, after the smoke from the previous puff had already condensed.

A reverse smoking machine[10] was utilized to produce smoke puffs. This type of smoking machine allows a stream of smoke to be delivered to the system under study at a slight positive pressure. Standard smoking conditions commonly used with the conventional vaccum type smoking machine are possible with this reverse machine. In this work, University of Kentucky reference cigarettes, IRI[11,12] were used to produce puffs of smoke. Standard 35 ml puffs of 2 sec duration were given to the roots of onion seeding after 10 min interval. The air in that system for smoking chamber was designed in order to deliver a near rectilinear puff profile. Puff volume adjustment was made by total smoke volume delivered by the cigarettes. This voume is generally greater than the volume of the air delivered to the cigarette in the smoking chamber. About 24 h after smoke treatment the root tips were fixed in 95% ethanol:acetic acid (3:1) at room tempera-

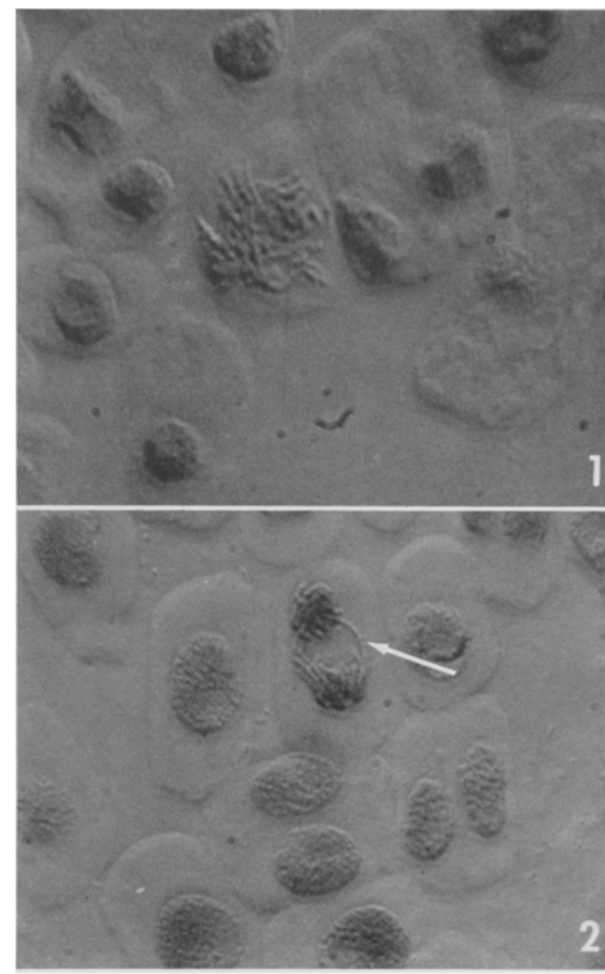

Fig. 1. 'Stickiness' of the chromosomes, in a dividing cell of a root-tip exposed to 20 puffs of cigarette smoke. ×1170.

Fig. 2. A dividing cell of a root-tip treated with 25 puffs of cigarette smoke. Note a lagging chromosome (see arrow) forming a bridge during anaphase. ×1039.

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Effect of tobacco smoke puffs on mitotic index in root tips of *Allium cepa* (onion) after 20 h

Tobacco smoke puffs	0 (Control)	5	10	15	20	25
Mitotic index	0.08	0.062	0.058	0.052	0.05	0.05

ture. They were hydrolyzed 24 h after fixation, with 1N HCl at 60 °C for 2 min. This was followed by several washings with distilled water. They were then stored in 45% acetic acid, until squashes were made in acetocarmine from the apical 2 mm. The percentage of cells in mitosis in each experiment were determined on the basis of a minimum of 1000 cells. Observations on the percentage of mitotic abnormalities were recorded.

Effect of tobacco smoke on general morphology. In untreated seedlings the cells had well-defined nuclei and the mitotic division was normal in 95% cells. Cells exposed to the tobacco smoke showed little evidence of injury. Only a few cells treated with tobacco smoke were damaged, as evidenced by blebbing of the cytoplasm and slightly pycnotic nuclei in the stained preparations. Treated cells, in general, were highly vacuolated.

Effect of tobacco smoke on mitotic index. In controls, about 9% of the cells were in mitosis, there being a variation from 7–10% in various samples. Treatment with tobacco smoke led to a decrease in mitotic index; magnitude of the effect varied with the number of puffs to which seedlings were exposed. Control cells showed a mitotic index of 0.09 while roots treated with 25 puffs showed an index of 0.05. Table I summarizes mitotic indices of onion root-tips exposed to different numbers of tobacco smoke puffs.

Effect of tobacco smoke on chromosomal abnormalities. Tobacco smoke also induced mitotic abnormalities. The percentage of irregularities increased with increasing number of puffs. Five 35-ml puffs induced abnormalities in only 10% of dividing cells while 10, 15, 20 and 25 puffs caused 38%, 47%, 58% and 60% irregularities, respectively, in the dividing cells. In general, 'stickiness' of chromosomes (Figure 1), and lagging chromosomes (Figure 2) in the equatorial plate were observed.

NAKANISHI et al.[3] while working with human epithelial lung cells treated with cigarette smoke condensate observed a decrease in average chromosome number to 76, from a modal value of 77 for the chromosomes in untreated cells. This finding was attributed to the appearance of dicentric chromosomes in some of the passages. BOUCHARD and MAY[13] observed an increased number of mitotic abnormalities in mouse lung fragments that had been bathed for 24 h in a smoke condensate solution. Later investigations[14–16] also found that cigarette smoke condensate has profound influence upon mammalian cells. Yet with a very few exceptions[17–20] which are perhaps best exemplified by chronic granulocytic leukemia in humans, the observed chromosomal anomalies commonly found in cancer cells show no consistent pattern. The present investigation is another approach used to ascertain the feasibility of utilizing plant tissues as a quick and sensitive bioassay for tobacco smoke. Vacuolization of the cytoplasm, pycnotic nuclei and other mitotic abnormalities were seen 24 h after exposure to cigarette smoke. Other mitotic irregularities include occasional 'stickiness' of chromosomes at metaphase (Figure 1) and bridging at anaphase and telophase (Figure 2). Thus, the present investigation clearly demonstrates that one can detect similar cytological responses in plants and animals exposed to tobacco smoke. A study of tobacco smoke and its constituents on plant tissues might lead to a better understanding of their mechanism of action. We are currently investigating the effect of different subfractions of tobacco smoke and results will be reported subsequently.

Zusammenfassung. Es wurden Untersuchungen der Wirkung des Tabakrauches auf mitotische Fehler bei *Allium-cepa*-L.-Sämlingen gemacht und dabei eine Abnahme des mitotischen Indexes und induzierte mitotische Missbildungen gefunden.

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Influence of Prior Exposure of Male Khapra Beetle (*Trogoderma granarium*) to Female Sex Pheromone on Their Mating Ability

It has been suggested that by saturating the field atmosphere with female sex pheromone or its synthetic analogues, male orientation to female could be effectively disrupted[1–3]. Experimental proof in support of this has been provided by GASTON et al[4]. They maintained in the field a high concentration of *Trichoplusia ni* pheromone and showed that under these conditions males were unable to orient towards virgin females held in traps. Sensory adaptation and/or absence of definite odour trail have been suggested as possible reasons for this phenomenon. It is however, also possible that the males were exhausted as a result of high excitation due to stimulation by the pheromone, and were unable to fly towards the trapped females. It is then likely that such exhaustion would impair their mating ability. We therefore investigated the effect of possible pheromone-induced fatigue on the ability of males to mate, using the khapra beetle, *Trogoderma granarium* Everts as test insect.

Five-day-old virgin males were confined in air-tight glass specimen vials (8 × 2.3 cm ⌀) each containing a filter paper disc impregnated with 0.2 ml of pheromone extract[5] (equivalent of 50 females' secretion over a 2-day-period). After 48 h these males were removed from the vials and paired in batches of 10 with an equal number of 1-day-old virgin females and were held in plastic containers (6 × 6 cm ⌀) with broken wheat as oviposition medium. Untreated males similarly paired served

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