A NEW METHOD FOR ISOLATION OF *NEMATOSPORA CORYLI* PEGLION FROM VECTOR BUGS

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Summary

A new method was developed to isolate *Nematospora coryli* Peglion from vector bugs. The method involved the use of apple fruits, which were previously fed by infested bugs. The fungus was isolated from *Acrosternum heegeri* Fieb., *Brachynema germari* Kol. and *Lygeaus panderus* Scop. by this method. The results were superior to those reported previously by other authors.

Introduction

Since the association between the bugs and the stigmatomycosis was known, numerous researchers attempted to isolate the pathogen from the vectors. WINGARD (1925) failed to isolate the fungus from the bug, but claimed the failure was due to the improper technique. FAWCETT (1929) was not able to obtain *N. coryli* from *Leptoglossus zonatus* whereas he reported the fungus could be transmitted readily by this insect. WEBER (1933) made dilution plates of the mouth parts of a dozen individual bugs and obtained no *Nematospora* sp. CHUPP and SHERF (1960) claimed that "attempts were made to isolate the organism from the insect, but the results always were negative", while before them LEACH and CLULO (1943) reported that they isolated the fungus from green stink bug, but they did not mention the method used.

In recent years FOSTER and DAUGHERTY (1969) and also CLARKE and WILDE (1970) were able to isolate *N. coryli* from transmission bugs and they described the method used clearly. As the writers were studying the host range of *N. coryli* in nature, they found some apple fruits which were attacked by bugs, and from these fruits they isolated *N. coryli* readily. So, based on this observation the following method was developed.

Materials and Methods

Insects are collected and caged in a container. The cage should not be big, but must have enough space for the bugs, and in this case, a container with the dimensions $10 \times 10 \times 20$ cm is adequate for 10 bugs. For the observation of the insects inside the cage, it is preferable that the container be made

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from a transparent material. To reduce the mortality of bugs, some parts (4×4 cm) of walls and lid must be replaced by Saran^R screen. The container with vectors is placed in a room maintained at 27°C and 80% RH (WILDE, 1968).

The insects are kept first without food for a maximum of 24 hr and then fed on healthy apple fruits, which have been surface sterilized with 70% alcohol. The number of fruits should be proportionate to the number of bugs and space of cage, and for a container of 10×10×20 cm, 2–3 fruits 5 in diam., is sufficient. There s not much difference between the apple varieties for the feeding, but it is preferable to choose sweet, green or yellowish fruits which are not hard.

After 48 hr feeding the fruits are taken out from container, peeled about 1 cm^2 at each puncture with sterilized scalpel. Then a part of the pulp of the fruit is cut at puncture and plated on CMA. To obtain a good result, it is better to take 3–4 plates and to inoculate each plate at 4–5 points. Inoculated plates are incubated at 27–30°C for 5–7 days, and then the grown fungus is subcultured to obtain a pure culture of *N. coryli*.

Result and Discussion

By the above mentioned method the writers were able to isolate readily *N. coryli* from *Acrosternum heegeri* Fieb., *Brachynema germari* Kol. and *Lygeaus panderus* Scop. which were collected from Rafsanjan (pistachio growing area of Iran). If the aim is to determine whether bugs are infested and if they feed well on apple fruit, this method is more sure than those used by others, because it is not necessary to kill the bugs (FOSTER and DAUGHERTY, 1969; CLARKE and WILDE, 1970) and by using the method usually a pure culture of fungus is obtained (WEBER, 1933). Moreover, streptomycin sulfate, which is used by FOSTER and DAUGHERTY (1969) and CLARKE and WILDE (1970) as bacteriostatic is not effective against all bacteria (DOMSCH and SCHWINN, 1965).

References

CHUPP, C. and SHERF, A.F. 1960. Yeast spot of lima bean, Nematospora coryli Peglion. - Vegetable diseases and their control: 148–150. Ronald Press Company, New York.

CLARKE, R.G. and WILDE, G. 1970. Association of the green stink bug and the yeast-spot diseases organism of soybeans. I. Length of retention, effect of molting, isolation from feces and saliva. - J. econ. Ent., **63** (1): 200–204.

DOMSCH, K.H. und SCHWINN, F.J. 1965. Nachweis und Isolierung von pflanzenpathogenen Bodenpilzen mit selektiven Verfahren.– Zentbl. Bakt. Parasit Kde, Suppl. I.: 461–485.

FAWCETT, H.S. 1929. Nematospora on pomegranates, citrus, and cotton in California. – Phytopathology, **19**: 479–482.

FOSTER, J.E. and DAUGHERTY, D.M. 1969. Isolation of the organism causing yeast-spot disease from the salivary system of the green stink bug.– J. econ. Ent., **62**: 424–427.

WEBER, G.F. 1933. Occurrence and pathogenicity of Nematospora spp. in Florida. – Phytopathology, **23**: 384–388.

WILDE, G. 1968. A laboratory method for continuously rearing the green stink bug. – J. econ. Ent., **61** (6): 1763–1764.

WINGARD, S.A. 1925. Studies on the pathogenicity, morphology, and cytology of Nematospora phaseoli - Bul. Torrey Bot. Club., **52**: 249–290.