Agriculture, Ecosystems and Environment, 15 (1986) 231—239
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

ASSESSMENT OF THE FUNGUS *CLADOSPORIUM OXYSPORUM* (BERK. AND CURT.) AS A POTENTIAL BIOCONTROL AGENT AGAINST CERTAIN HOMOPTERA

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(Accepted for publication 30 September 1985)

ABSTRACT

Samways, M.J. and Grech, N.M., 1986. Assessment of the fungus *Cladosporium oxysporum* (Berk. and Curt.) as a potential biocontrol agent against certain Homoptera. *Agric. Ecosystems Environ.*, 15: 231—239.

Cladosporium oxysporum (Berk. and Curt.) was isolated from *Planococcus citri* (Risso). In the laboratory, the pathogen was grown in submerged culture and then applied to *P. citri*, *Pseudococcus longispinus* (T.T.), *Coccus aethiopicus* De Lotto and *Trioza erytreae* (del Guercio), causing death and hyphal growth in all four. Applications in the field had considerable initial impact upon populations of *Toxoptera citricidus* (4 trials) and *T. erytreae* (1 trial). An unidentified toxin may be more instrumental in causing death than direct hyphal growth.

INTRODUCTION

The genus *Cladosporium* was traditionally thought of as being saprophytic (Ellis, 1971), although in recent years pathogenicity to insects has been recorded (Zi-Chao and Zhuang-Tu, 1980; Bellotti, 1983; Samways, 1983). This paper investigates *C. oxysporum* (Berk. and Curt.), which caused epizootics in populations of the mealybug *Planococcus citri* (Risso) and the aphid *Aphis gossypii* Glover on guava trees (*Psidium guajava* L.) in Eastern Transvaal (Samways, 1983). This paper reports on the species of insects found in association with *C. oxysporum* in nature, methods of laboratory culture of the fungus and its application in the laboratory and field against various insect species.

TAXONOMY AND IDENTIFICATION OF THE PATHOGEN

The fungus producing a profuse growth of conidiophores on the exterior of *P. citri* during epizootics (Fig. 1a) was determined by Dr. G.C.A. van der

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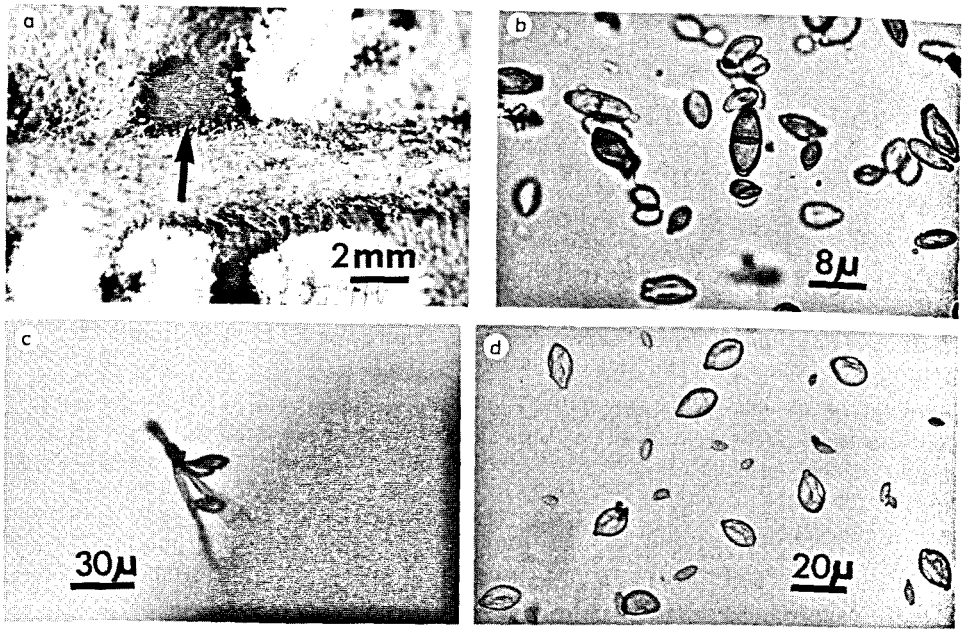

Fig. 1. (a) A dead *Planococcus citri* (arrow) covered with a profuse mycelial growth of *Cladosporium oxysporum*. The other mealybugs, which are white and resting against the median vein of a guava leaf, died from the same disease a few days later. (b) Normal conidia from a plate culture. (c) Conidia and conidiophores growing on the exterior of a moribund insect. (d) Small and large conidia characteristic of the entomopathogenic phase of this fungus.

Westhuizen of the Plant Protection Research Institute, Pretoria, as *C. oxysporum*. A slant culture which was sent to the Commonwealth Mycological Institute, London, was also determined by Dr. D.W. Minter as *C. oxysporum* (Fig. 1b). However, spores different from those of *C. oxysporum* (Bievre, 1981) have been seen on insect cadavers (Fig. 1c, d). Because of the presence of these spores, and as the fungus was recorded here as an entomopathogen, which is outside its normal host range (Ellis, 1971), we are only provisionally referring to it as *C. oxysporum*.

MATERIALS AND METHODS

Laboratory culture of the pathogen

Spores of *C. oxysporum* were removed from field-collected cadavers and moribund individuals of *P. citri*, and were cultured on a solid medium and also submerged in a liquid medium. The initial cultures on potato dextrose agar (PDA) showed poor growth. Other media were tested, and a modified PDA containing whole-insect or shrimp-shell extracts proved successful.

The addition of purified chitin (5 g l^{-2}) gave similar results, suggesting that chitin in the insect and shrimp extracts contributed to growth. Submerged cultures were best for bulk-culture. The medium was prepared by homogenising 0.5 kg of thoroughly washed shrimp shells in 1 l of water. Then 5 ml of the extract was added to 1 l of Czapek's dox broth. A conidial suspension from cultures on solid media (chitin-amended PDA) was used to inoculate the liquid media, which were maintained in 250-ml Erlenmeyer flasks at 25 ± 2°C in a 15 h/9 h light/dark cycle.

Laboratory application

Target organisms (*P. citri*, *Pseudococcus longispinus* (T.T.), *Coccus aethiopicus* De Lotto and *Trioza erytreae* (del Guercio)) were established on potted navel-orange trees (*Citrus sinensis* (L.) Osbeck). A conidial suspension of *C. oxysporum* was applied to the plants at a spore concentration of 1.5×10^8 spores ml^{-1}. Water sprays were used as controls. Target insects were examined daily for fungal infection.

Application in the field

The spore concentration in the sprays was 4×10^8 conidia ml^{-1}. A wetting agent, 0.1% Tween®, was also added. Controls were of two types: (a) the same composition (i.e. contained an equivalent amount of agar and wetter) but without any *C. oxysporum*; (b) distilled water. Whole, marked shoots and the area immediately surrounding them were sprayed. All sprays on all surfaces were applied to the point of run-off by a hand sprayer. Insect levels were assessed by counting the number of individuals per distal 20 cm of shoot every 2 days initially, and at longer intervals later, for a minimum period of 1 month.

RESULTS

Naturally occurring epizootics and enzootics

Besides causing epizootics among *P. citri* and *Aphis gossypii* on guava trees (Samways, 1983), *C. oxysporum* could decimate a population of *Toxoptera citricidus* (Kirk.) on lemon trees (*Citrus limon* (L.) Burm. f.) and *A. gossypii* on cotton plants (*Gossypium* spp.). In addition, some adult individuals of *Aonidiella aurantii* (Mask.), *Chrysomphalus aonidum* (L.), *P. longispinus* and *C. aethiopicus* were infected, as were nymphs of *T. erytreae*. All these results were found in the Eastern Transvaal lowveld.

Host range of Cladosporium oxysporum

C. oxysporum applications in the laboratory caused mortality of *P. citri*, *P. longispinus*, *C. aethiopicus* and *T. erytreae*.

Larvae of the Lepidoptera *Papilio demodocus* Esp. and *Cryptophlebia leucotreta* (Meyr.) were unaffected when inoculated by a conidial spray of *C. oxysporum* (3.2×10^8 spores ml^{-1}) in the laboratory.

Despite natural contact with the fungus in the field, no infection was observed in the ants *Anoplolepis custodiens* (Smith) and *Pheidole megacephala* (F.), or in the coccinellids *Scymnus* spp., *Exochomus flavipes* Thunb., *Cheilomenes propinqua* (Muls.), *C. lunata* (F.) and *Chilocorus angolensis* Crotch. Spores were also found on the body surface of apparently healthy *A. custodiens* and of unidentified Muscidae feeding on honeydew excreted by *P. citri*.

Field applications against Toxoptera citricidus

Readily available citrus insects, rather than those of guavas, were used as targets in these trials. Initial applications of *C. oxysporum* in the summer (January) resulted in profuse hyphal growth on the cadavers of *T. citricidus* (Fig. 2a, b). Prior to death, hyphal growth appeared on the intersegmental areas between sclerites, at the base of the femur and on the antennae.

In a second trial during the dry winter period (13 May 1983), 1 day after treatment with *C. oxysporum*, the *T. citricidus* population dropped to 8.3% of its former level (Fig. 3). With the agar-plus-wetter control, it dropped to 34.9% and with the water control to 72.4%. With the hardening of the flush, many aphids left the water-treated shoots, hence the dramatic drop in this particular population after 16 May (Fig. 3). The first hyphal growth appeared on 23 May, 9 days after the treatment. Aphids on 40% of the *C. oxysporum*-treated twigs showed sporulation. However, some sporulation in aphids on one of the twigs in the agar-plus-water control began on the same date, illustrating either some cross-infection or the appearance of endemic *C. oxysporum*.

A third trial, also in May, showed similar results. One day after application, the *C. oxysporum*-treated aphid levels dropped to 49.8%, the population treated with the agar-plus-water dropped to 67.3%, while the population treated with water actually increased by 15.6%.

A fourth trial was carried out from November 1982 to January 1983, at a time when there are normally heavy summer rains (Fig. 4). An exceptionally hot and dry year made conditions abnormal. After application of the agar-plus-wetter control, the aphid population increased by 43.4% while the pathogen-treated populations dropped to 82.8%. These results are similar to those in Fig. 3, with aphids reproducing so rapidly that the population reductions due to spray applications were compensated for by the addition of the newborn.

Figure 4 shows how the pathogen-treated population followed that of the agar-plus-wetter control, only at lower levels. Initially, the pathogen appeared to severely reduce the levels, the overall population fluctuations being regulated by external factors.

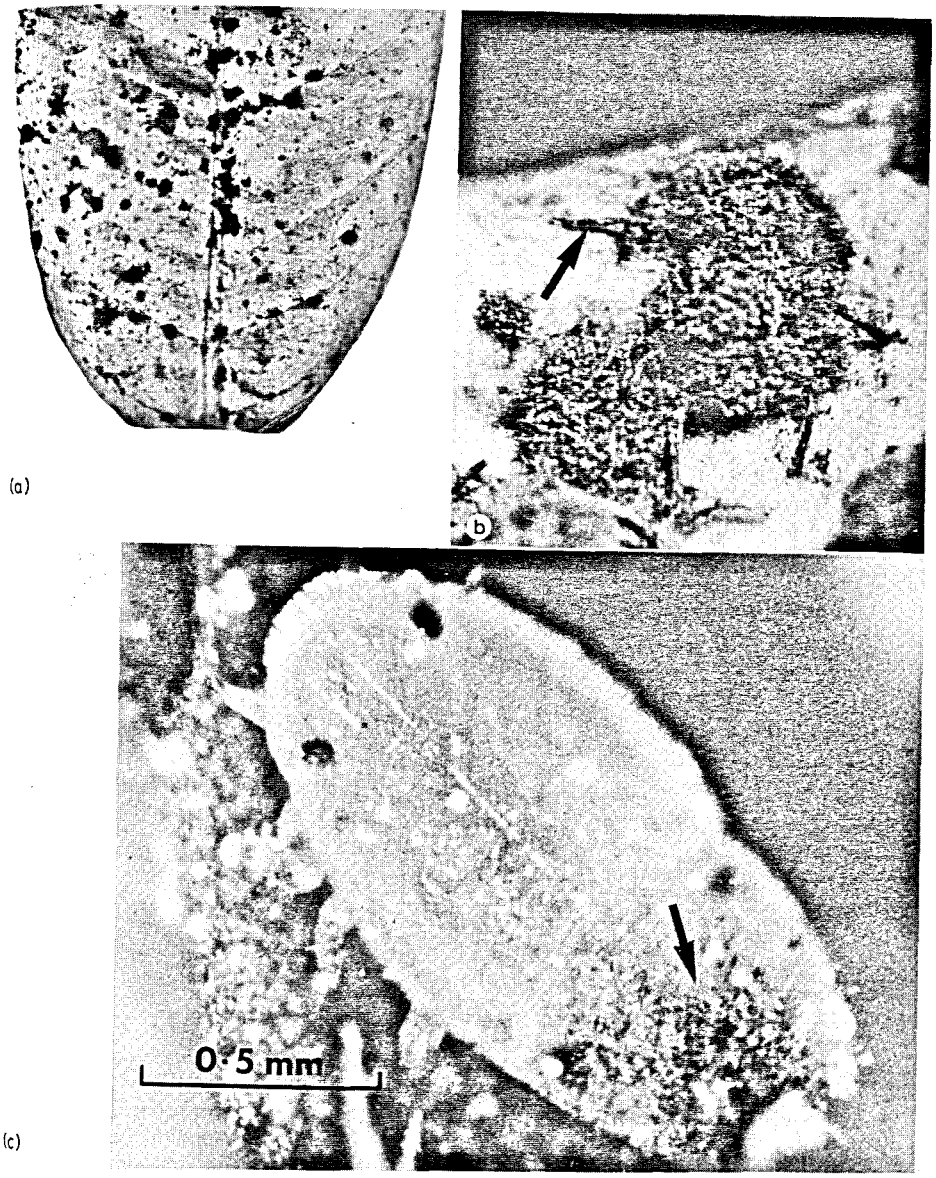

Fig. 2 (a) Total mortality of *Toxoptera citricidus* in the field, 34 days after high-volume application of conidia of *Cladosporium oxysporum*. (b) Close-up of dead aphids smothered with hyphae and asexual sporulation structures with only the insect's legs (arrow) still visible. (c) Hyphae (arrow) on and between tergites of a last-instar nymph of *Trioza erytreae* in the laboratory, 14 days after high-volume application of conidia of the pathogen.

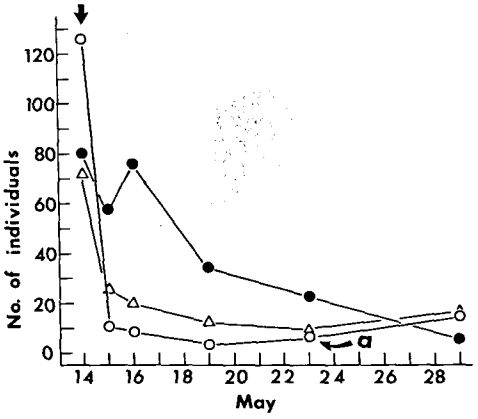

Fig. 3. Decreasing winter population levels of the aphid *Toxoptera citricidus* after field application (arrow) of conidia of *Cladosporium oxysporum* (open circles), a control broth lacking the pathogen (triangles) and water (closed circles). The point 'a' marks the first signs of actively growing hyphae among the aphid populations. The standard error bars are omitted for clarity (see Fig. 5).

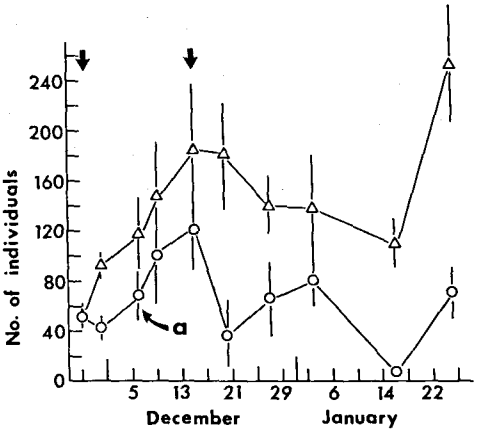

Fig. 4. Fluctuating summer population levels of the aphid *Toxoptera citricidus* after two applications (arrows) in the field of conidia of *Cladosporium oxysporum* (circles) and a control broth lacking the pathogen (triangles). Point 'a' marks the first sign of activity growing hyphae among the aphid population.

Influence of physical conditions

Figures 3 and 4 show that external hyphae first appeared 9 days after application. A characteristic feature of the application against *T. citricidus* on citrus was the way in which some shoots showed high aphid mortality and yet on others, the aphid populations were little affected. This is illustrated

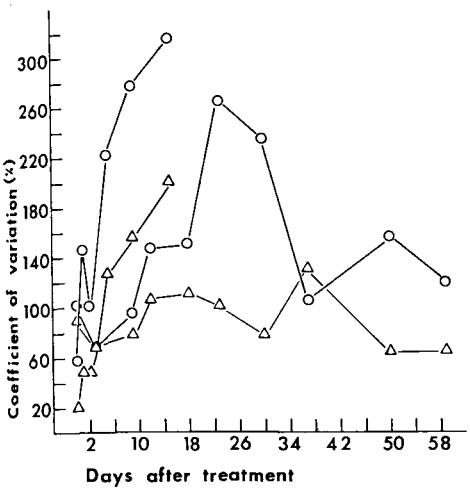

Fig. 5. Changes in the coefficient of variation for the field population of *Toxoptera citricidus*, illustrated in Figs. 3 and 4, after being treated with conidia of *Cladosporium oxysporum* (circles) and an agar-plus-wetter control lacking the pathogen (triangles).

in Fig. 5 which shows a steep rise in the coefficients of variation for field populations of *T. citricidus*.

In all cases where the aphids were entirely eliminated, the host shoots were sheltered and/or in the shade. In contrast, on all shoots that were exposed to the sun, the populations escaped the disease, suggesting that the degree of exposure to physical conditions partially determined disease establishment.

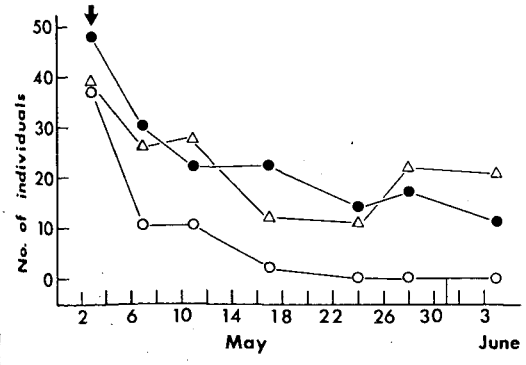

Fig. 6. Decreasing winter population levels of the psylla *Trioza erytreae* after applications (arrow) of conidia of *Cladosporium oxysporum* in the field (open circles), a control broth lacking the pathogen (triangles) and water (closed circles). Standard error bars are omitted for clarity.

Field applications against Trioza erytreae

Psylla levels dropped after application of *C. oxysporum*, and also after application of the control (Fig. 6). Four days after treatment, the 2 controls did not differ significantly, but the pathogen-treated population was significantly lower ($P < 0.01$). Thereafter, the pathogen-treated population was always significantly lower ($P < 0.01$), and dropped to zero 21 days after treatment. Most of the psylla died without signs of hyphal growth.

DISCUSSION

Laboratory applications of *C. oxysporum* gave good hyphal growth on various Homoptera. Establishment of the pathogen was not so obvious in field situations. Results were similar for aphids and psylla, with high initial mortality and later appearance of external mycelia. The agar-plus-wetter control produced a higher initial mortality than the distilled water control. The initial mortality might occur because of the physical effect of the spray suspension in addition to a toxic compound produced by the fungus. In a first step, *C. oxysporum* is able to establish on honeydew and insect cadavers. In a second step, the fungus could infect aphids which would die either as a result of direct hyphal growth or by the effect of a toxin produced by the mycelia growing on cadavers nearby.

Eradication of insects on treated shoots was achieved only in nymphs of *T. erytreae*. The leaf-curling commonly induced by young *T. erytreae* provided a microclimate which favoured fungal development. Indeed, *C. oxysporum* was often present within the curled-up leaves on cadavers of various insects, possibly providing a reservoir for later enzootic and epizootic activity.

On sun-exposed shoots, some aphids survived the treatment and later gave rise to large populations on the growing tip. By contrast, populations on sheltered shoots usually died out.

High populations of *P. citri* and *T. gossypii* on the guavas were sustained by a protective mutualism with ants (Samways, 1983) which kept out natural enemies. Such populations, associated with high honeydew levels, were a perfect stage for density-dependent *C. oxysporum* activity.

The use of *C. oxysporum* to control *T. erytreae* on citrus in southern Africa is not promising. This insect is an efficient vector of the serious greening disease (as yet unnamed); control, therefore, aims at maintaining the insect at a low level. As *C. oxysporum* is density-dependent, it is unsuitable for the control of low-level psylla populations. It would therefore be impractical to attempt complete psyllid suppression. This is particularly the case in spring, when *T. erytreae* is on the increase and the atmospheric conditions are not suitable for the pathogen. These spring-time weather limitations also apply to *T. citricidus*.

In conclusion, applications of *C. oxysporum* conidia have drawbacks as an

economic control measure for citrus insects in southern Africa. However, isolation and development of a possible toxin may have economic potential, as may suspensions of conidia, against high homopteran infestations in the humid tropics.

ACKNOWLEDGEMENTS

Special thanks to Anne marie Oosthuizen for technical assistance.

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