Does a vascular fungus of tomato induce a defence or a change in host plant quality that also affects the oviposition of spider mites?

Jongebloed, P.H.J.; Elgersma, D.M.; Sabelis, M.W.

Does a vascular fungus of tomato induce a defence response or a change in host plant quality that also affects the oviposition of spider mites?

P.H.J. Jongebloed[a,1], D.M. Elgersma[b] and M.W. Sabelis[a]

[a]*Department of Pure and Applied Ecology, University of Amsterdam, Amsterdam, Netherlands*
[b]*Department of Molecular Cell Biology, University of Amsterdam, Amsterdam, Netherlands*

ABSTRACT

Jongebloed, P.H.J., Elgersma, D.M. and Sabelis, M.W., 1992. Does a vascular fungus of tomato induce a defence response or a change in host plant quality that also affects the oviposition of spider mites? *Exp. Appl. Acarol.*, 16: 227–236.

It has been suggested that previous infection by a vascular fungus causes induced resistance against two-spotted spider mites. To test the generality of this phenomenon, a series of experiments was carried out using two lines of tomato, differing only in resistance against *Fusarium*. In addition, tests were done in order to see whether the defense response against the fungus also affects the phytophagous mite directly. Inoculation of tomato plants with a vascular fungus (*Fusarium oxysporum* f.sp. *lycopersici* race 1) prior to infestation with spider mites caused a decrease in the rate of oviposition of two-spotted spider mites (*Tetranychus urticae*) on a *Fusarium*-susceptible line, but only when plants were moderately to severely wilted. Spider mite oviposition did not change significantly on a previously inoculated *Fusarium*-resistant line.

As *Fusarium* causes vascular occlusion and wilting of the plants, drought stress was experimentally induced to determine its influence on the reduction of oviposition. Drought caused a significant reduction in spider mite oviposition. We conclude that the effect of previous *Fusarium*-inoculation on spider mite oviposition is primarily due to the fungus affecting the quality of the host plant (including the effect it may have on the composition of defensive compounds), rather than due to the stimulation of the defense system of the plant. Since *Fusarium* seals off the xylem vessels, thereby causing wilting of susceptible plants, the reduction in mite oviposition may well be due to drought stress in the leaves, rather than due to the production of phytoalexins.

Correspondence and present address: P.H.J. Jongebloed, Research Institute for Plant Protection (IPO-DLO), Postbox 9060, 6700 GW Wageningen, Netherlands.

INTRODUCTION

Induced responses against plant pathogens are commonly found in both natural and agricultural systems. Inoculation of plants with non-pathogenic microorganisms or with an avirulent strain of a fungus, virus or bacterium may induce resistance against a pathogenic strain of the same pathogen when introduced later (Kuc, 1982; Biles and Martyn, 1989). These (avirulent) inducers probably trigger a defensive system that protects the plant against virulent challengers. For instance, restricted infection of cotyledons with certain pathogens may induce persistent, broad-spectrum protection against subsequent infections by other pathogens. Restricted inoculation of cucumber (*Cucumis sativus* L.) with the anthracnose fungus, *Colletotrichum lagenarium* (Pass.) Ell. & Halsted, provides systemic protection against the same fungus (Kuc et al., 1975) and against at least 12 other plant pathogens (Kuc, 1982, 1983, 1987). In addition, other host changes caused by herbivore damage lead to a reduction in subsequent herbivore abundance (c.q. Rhoades, 1983; Karban, 1985; Faeth, 1987).

Karban et al. (1987), working with the vascular fungus *Verticillium dahliae* Kleb. and the spider mite *Tetranychus urticae* Koch on cotton (*Gossypium hirsutum* L.), found that previous inoculation of cotton with *V. dahliae* resulted in a reduction in oviposition by *T. urticae*. The authors assumed that *V. dahliae* induced resistance in cotton plants against *T. urticae*. Since they used only a *Verticillium*-susceptible cotton line, it was not possible to determine whether the effect on mite fecundity is primarily due to a change in food quality or to defensive actions of the plant.

Such a distinction would have been possible if the experiments had been carried out on resistant lines as well. Hence, in the present study herbivore performance was investigated on both fungus-resistant and fungus-susceptible lines in an attempt to analyze the mechanisms underlying the interaction between the two types of plant enemies. We followed this experimental set up using another herbivore–fungus–host plant system than Karban et al. (1987). We chose *Lycopersicum esculentum* Mill., with the fungal pathogen *Fusarium oxysporum* Schlecht f.sp. *lycopersici* (Sacc.) Snyd. & Hans. and the herbivore *Tetranychus urticae* Koch. The reasons for studying this system are that (1) more information is available on the physiological mechanisms underlying defense against the fungus (vascular occlusion and the production of phytoalexins (Beckman, 1987)), (2) the availability of tomato lines that differ only in their resistance against the fungus and (3) each of the components of the system is easy to culture.

Preliminary experiments indicated that the induced effect on spider mite oviposition was the result of reduced plant quality rather than increased defensive effort. To understand better the underlying causes, it should be realized that *Fusarium* is a soil-borne pathogen, that penetrates the roots of to-

mato and rapidly invades the xylem vessels. In susceptible lines, the fungus is able to spread through the plant by hyphal growth from one vessel to another and by the transport of microconidia, which leads to wilting of the plants (Beckman, 1987). Degradation of plant tissue by the fungus in a susceptible line may lead to production of metabolites, toxic to the plant. These products and fungal metabolites, cause gummosis and tylosis (c.q. fusaric acid), leading to drought stress in the leaves of a susceptible line. For this reason a drought stress experiment was set up to study the effect of different drought stress levels on the rate of oviposition of *T. urticae*.

MATERIAL AND METHODS

Plant, fungal, and spider mite material

Prior to inoculation, tomato seeds of the line Moneymaker were sown in steamed soil in 90-cm plastic containers (30 seeds per container) and allowed to grow for 3 weeks in a climatic room at 25°C. Two lines of the cultivar were used: a *Fusarium*-resistant line (Moneymaker GRR 161) and a *Fusarium*-susceptible line (Moneymaker 32). The lines only differ in their resistance to *Fusarium* and were obtained from the Glasshouse Crops Research Institute, Littlehampton (UK).

The fungus strain used in the experiments was race 1 of *Fusarium oxysporum* f.sp. *lycopersici* (F.o.1, WCS 801). It was cultured on potato dextrose agar (PDA).

As herbivore, we used a tomato-adapted strain of *Tetranychus urticae*, obtained from the Glasshouse Crops Research and Experiment Station in Naaldwijk, the Netherlands. The strain was reared on tomato or bean leaves on moist cotton wool in a climatic chamber at 25°C, 65% RH, and a photoperiod of 16 h.

All experiments were performed in a climatic chamber at 25°C, 70% RH, and a 17-h photoperiod (300 W m^{-2}).

Preparation of fungal inoculum and inoculation of the plants

F.o.1 (race 1) was cultured on Czapec Dox (Oxoid) medium (3.34 g in 100 ml sterile tap water) and shaken on a reciprocal shaker at 23°C. Spore suspensions were made by filtering 5-day-old cultures through sterile glasswool to remove mycelial fragments, by washing the resulting spore suspension twice in sterile tap water by centrifugation and by subsequently adjusting it to a concentration of 10^{-7} spores ml^{-1}.

Twenty 3–5-week-old plants of both varieties were root-inoculated by dipping the roots in the spore suspension for 10 min. Twenty control plants of each variety were similarly treated with sterile water. After treatment, the plants were transferred to 9-cm plastic pots containing steamed soil.

Spider mite infestation of the plants

Twelve days before mite introduction, 500 adult females were placed on 20 bean leaves. After 24 h, the adult females were removed in order to obtain a synchronized wave of eggs. Females emerging from these eggs were used for the plant infections. Ten adult females were placed on one leaf of each plant. Migration to other leaves was prevented by a Tanglefoot® barrier around the leaf petiole. The infested leaves were taken off 3–4 days after mite introduction, the adult females were removed, and the number of eggs laid was determined. Three different periods of time between *Fusarium* inoculation and *Tetranychus* infestation were chosen (4, 7 and 11 days) in order to determine the relation between the degree of wilting (susceptible line) or the level of plant defense (resistant line) and mite fecundity.

Drought stress experiment

Tomato seeds were sown in steamed soil and kept in a climatic chamber at $25°C$, 70% RH, and 17 h light ($300 W m^{-2}$). Fourteen days after sowing individual plants were transferred to 1-l pots containing a nutrient solution (1 g Nutriflora® per l). The solutions were continuously aerated and weekly refreshed, and the roots were not exposed to light.

After 7 days the plants were exposed to drought stress. Polyethylene glycol (PEG 20 000, Breox) was added to the nutrient solution in order to reduce the osmotic potential. Four stress levels were created: 0%, 5%, 10% and 20% PEG. In contrast to Sumner et al. (1983), who used PEG 400 as a matricum, PEG 20 000 was used to avoid uptake by the plant. PEG 400 particles may be absorbed by the plant, causing physiological changes.

Seven days after imposition of the drought stress, five adult females were placed on one leaf (the second youngest leaf) of each plant. After 4 days the adult females were removed and the number of eggs deposited was determined.

The dry weight of the shoots and roots and also the influence of the different stress levels on leaf elongation were determined.

RESULTS

Fusarium experiment

Figure 1 (experiment 1) shows the daily egg production per adult female on plants on which mites were introduced 11 days after inoculation with *Fusarium*. The daily egg production on the susceptible *Fusarium*-infested line was significantly lower than on the resistant line and on non-inoculated plants ($P < 0.05$, one-factor ANOVA, comparison between groups using the Tukey least significant range test). The mites on the resistant plants did not show any significant changes in egg production after *Fusarium* infestation. The *Fu-*
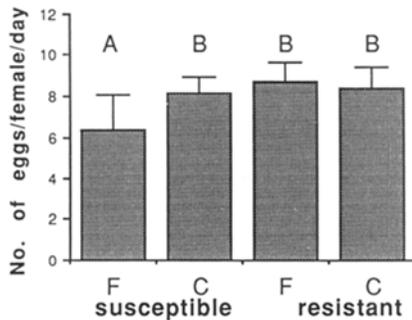
Fig. 1. Effect of previous *Fusarium oxysporum* f.sp. *lycopersici* (F.o.1) infection on the oviposition of *Tetranychus urticae* on tomato. Each bar represents the mean daily egg production per adult female on the third leaf of 20 tomato plants ($n=20$), and the standard deviation. The first two bars refer to the *Fusarium*-susceptible line, the last two bars to the *Fusarium*-resistant line. F refers to plants inoculated with F.o.1., C concerns untreated plants. Inoculation with F.o.1 or sterile water treatment was done at 16 DAP (days after planting), spider mite were introduced at 26 DAP and eggs were counted at 30 DAP. Bars with different letters are statistically different according to the Tukey multiple range test ($P<0.05$).
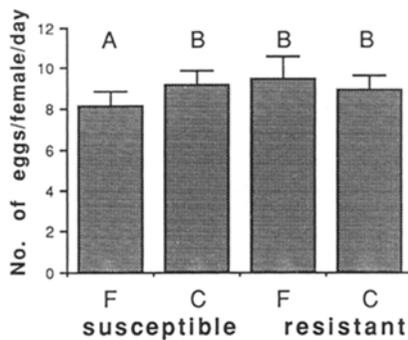

Fig. 2. Effect of previous inoculation with *Fusarium oxysporum* f.sp. *lycopersici* (F.o.1) on the oviposition of *Tetranychus urticae* on tomato. Each bar represents the mean daily egg production per mite ($n=20$) with the standard deviation. Inoculation with F.o.1 and sterile water treatment were done at 15 DAP (days after planting), spider mite were introduced at 24 DAP, and eggs were counted at 28 DAP. Bars with different letters are statistically different according to the Tukey multiple range test ($P<0.05$).

sarium-infested susceptible plants showed severe wilting symptoms, whereas the resistant plants did not show any wilting symptoms.

Oviposition was significantly reduced on plants of the *Fusarium*-susceptible line, when the mites were introduced 7 days after fungus inoculation, but this was not the case on plants of the *Fusarium*-resistant line ($P<0.05$, Tukey LSR-test; see Fig. 2 of experiment 2). The susceptible plants showed clear wilting symptoms, although the wilting was less severe than in experiment 1. The resistant plants did not show any wilting and had a healthy appearance.

In the case of a 4-day time interval between fungus inoculation and mite introduction no significant changes in ovipositional rate were observed (see Fig. 3 of experiment 3). The *Fusarium*-infected susceptible plants did not show any wilting symptoms.

In all three experiments a significant correlation was observed between degree of wilting and reduction in rate of oviposition rate of two-spotted spider mites on the *Fusarium*-suceptible line.

Drought stress experiments

The drought stress experiments showed a profound effect of severe drought stress (20% PEG, resulting in -1.03 MPa osmotic pressure in the root solution) on the rate of oviposition of *T. urticae* (see Fig. 4). The mite oviposition was significantly reduced ($P < 0.05$, Tukey LSR test) on plants treated with 20% PEG. The other drought levels did not cause significant changes in the oviposition of mites. Severe drought stress (20% PEG $= -1.03$ MPa)
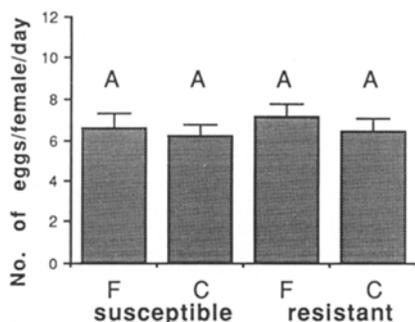

Fig. 3. Effect of previous inoculation with *Fusarium oxysporum* f.sp. *lycopersici* (F.o.1) on the oviposition of *Tetranychus urticae* on tomato. Each bar represents the mean daily egg production per adult female on the second leaf of 20 tomato plants with the standard deviation. Inoculation with F.o.1 and sterile water treatment were done at 37 DAP (days after planting), spider mite were introduced at 41 DAP, and eggs were counted at 44 DAP. Bars with different letters are statistically different according to the Tukey multiple range test ($P < 0.05$).

TABLE 1

Influence of drought stress on plant growth parameters and mite oviposition

Drought level	Shoot dry weight (g)	Root dry weight (g)	Leaf elongation (cm)
0 ($n = 24$)	4.74[a]	0.79[a]	0.328[a]
5 ($n = 14$)	4.43[a]	0.73[a]	0.270[a]
10 ($n = 24$)	3.42[b]	0.61[b]	0.219[b]
20 ($n = 19$)	1.53[c]	0.33[c]	0.071[c]

Different letters within a column means significantly different ($P < 0.05$, Tukey multiple range test).
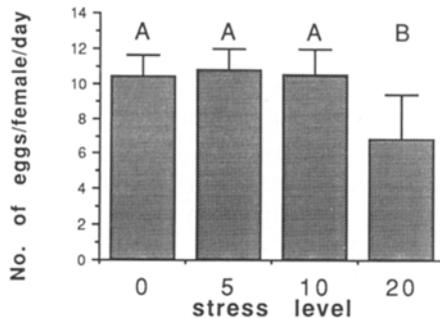
Fig. 4. Effect of different drought stress levels on the oviposition of *Tetranychus urticae*. Each bar represents the mean daily egg production on 14 to 24 plants. Bars with different letters are statistically different according to the Tukey multiple range test ($P < 0.05$).

caused a significant reduction in root dry weight and shoot dry weight compared to the other three treatments (Table 1).

Moderate drought stress (10% PEG $= -0.34$ MPa) also reduces shoot and root dry weight compared to control and 5% PEG. Leaf elongation (cm^{-1} day^{-1}) seems to be a good measure for the level of drought stress in the leaves (English-Loeb, 1989, 1990). At the highest stress level leaf elongation is significantly reduced. Moderate stress (10% PEG) also causes a diminished leaf growth compared to the control and 5% PEG treatment (Table 1). Root dry weight and shoot dry weight are strongly correlated ($P < 0.001$). Daily egg production of the spider mite is positively correlated with the shoot dry weight ($P < 0.001$), and with the leaf elongation ($P < 0.05$).

DISCUSSION

Infection of tomato plants with the vascular fungus induced a reduction in the rate of oviposition of *Tetranychus urticae* on the *Fusarium*-susceptible line, but only in the case of moderate or severe wilting of the leaves. The resistant line did not show any changes in mite oviposition after fungus infestation.

Before embarking on a discussion whether these results indicate induced resistance, it is important to point out that at least two different definitions of this term have been proposed. Karban and Myers (1989) include all possible plant-related causes in their definition of induced resistance. They define all induced responses leading to a decrease in herbivore preference or performance as induced resistance. Their definition does not distinghuish between a modification in host plant quality and changes in defensive allocations. In contrast, Kogan and Paxton (1983) define induced resistance as an active defense based on physical and/or chemical barriers and elicited by a

pathogen (or a non-pathogenic factor). The latter definition is generally accepted among plant pathologists.

We assume that the decrease in mite oviposition is mainly due to fungus-induced changes in host plant quality, rather than fungus-induced changes in the plant's defense. Host plant quality is affected by *Fusarium* by plugging of the xylem vessels and by the production of toxic metabolites, resulting in drought and nutrient stress of the leaves. This mechanism is not an example of induced resistance as defined by Kogan and Paxton (1983), although according to the definition of Karban and Myers (1989) the results presented by us would still fit the term induced resistance. Our drought stress experiments clearly showed a negative relationship between severe drought stress (20% PEG) and the performance of *T. urticae* as found by others (e.g. Mellors and Propts, 1983; Sumner et al., 1983; Oloumi-Sadeghi et al., 1988; McQuate and Connor, 1990a,b). The lower drought levels did not cause significant changes in mite oviposition. Thus, our results are not in agreement with White (1969, 1974, 1976, 1984), Lewis (1979) and Hollingworth and Berry (1982) who reported a stimulation of insect outbreaks on drought-stressed plants nor with English-Loeb (1989, 1990) who found a non-linear response of drought stress on mite performance. Such a non-linear relationship was postulated earlier by White (1984) and Mattson and Haack (1987a,b) in extreme stress situations, in which the plant is so badly damaged that it has become unsuitable for herbivores. At low drought stress the plant is more suitable for herbivores since it is both more nutritious and less well defended. Mattson and Haack (1987a) state that drought may have an effect on nitrogen content and the production of defensive compounds. It should therefore be realized that the experiments presented in this paper do not exclude possible changes in defensive allocations. Changes in host plant quality caused by drought stress may be the cause of a changed investment in defensive plant compounds. The production of secondary metabolites (phytoalexins), after penetration of a pathogenic fungus, is much higher on a incompatible interaction (resistant line) than on a compatible interaction (susceptible line). This means that spider mite oviposition is expected to be lower on the *Fusarium*-resistant line than on the *Fusarium*-susceptible line, if secondary metabolites have an important effect on egg production. However, our experiments showed that oviposition was higher on the resistant line, making it unlikely that the production of phytoalexins significantly affected the oviposition.

In our system the vascular fungus and the spider mite triggered different defense systems. *Fusarium* is unable to immunize the tomato line Moneymaker against spider mite damage, as was found by Karban et al. (1987) in their experimental setup using another host plant. Previous mite-feeding on the cotyledons did not affect the disease expression of subsequent introduced *Fusarium* (Jongebloed, unpublished results), as found by Karban et al. (1987)

for *Verticillium* infecting cotton. Ajlan and Potter (1991) found that restricted infection with the anthracnose fungus *Colletotrichum lagenarium* did not induce resistance against the two-spotted spider mite, fall armyworm, and melon aphids. They also showed in reciprocal tests, that previous feeding damage caused by spider mites or fall armyworms did not induce systemic resistance to *C. lagenarium*.

It seems reasonable to suggest that different defense systems are acting against the vascular fungus and the phytophagous spider mite, as found by Aljan and Potter (1991), working with another fungus–herbivore–host plant system. *Fusarium* causes damage in the xylem vessels, spider mite damage is located on the leaves. The different locations of the pathogen and the herbivore in and on the plant may be the main reason for the fact that different defense systems are acting against these two organisms.

REFERENCES

Ajlan, A.M. and Potter, D.A., 1991. Does immunization of cucumber against anthracnose by *Colletotrichum lagenarium* affect host suitability for arthropods? Entomol. Exp. Appl., 58: 83–91.

Beckman, C.H., 1987. The Nature of Wilt Diseases of Plants. A.P.S. Press, The American Phytopathological Society St. Paul, MN.

Biles, C.L. and Martyn, R.D., 1989. Local and systemic resistance induced in water melons by Forma Specialis of *Fusarium oxysporum*. Phytopathology, 79: 856–860.

English-Loeb, G.M., 1989. Nonlinear responses of spider mites to drought-stressed host plants. Ecol. Entomol., 4: 45–55.

English-Loeb, G.M., 1990. Plant drought stress and outbreaks of spider mites: a field test. Ecology, 71: 1401–1411.

Faeth, S.H., 1987. Community structure and folivorous insect outbreaks: The roles of vertical and horizontal interactions. In: P. Barbosa and J.C. Schultz (Editors), Insect Outbreaks. Academic Press, New York, NY, pp. 135–171.

Hollingworth, C.S. and Berry, R.E., 1982. Two-spotted spider mite (Acari: Tetranychidae) in peppermint: population dynamics and influence of cultural practices. Environ. Entomol., 11: 1280–1284.

Karban, R., 1985. Resistance against spider mites in cotton induced by mechanical abrasion. Entomol. Exp. Appl., 37: 137–141.

Karban, R. and Myers, J.H., 1989. Induced responses to herbivory. Annu. Rev. Ecol. Syst., 20: 331–348.

Karban, R., Adamchak, R. and Schnathorst, W.C., 1987. Induced resistance and interspecific competition between spider mites and a vascular wilt fungus. Science, 235: 678–680.

Kogan, M. and Paxton, J., 1983. Natural inducers of plant resistance to insects. In: P. Hedin

(Editor), Plant Resistance to Insects. Am. Chem. Soc. Symp. Series, Washington, DC, pp. 153–171.

Kuc, J., 1982. Induced immunity to plant disease. Bioscience, 32: 854–869.

Kuc, J., 1983. Induced systemic resistance in plants to diseases caused by fungi and bacteria. In: J.A. Bailey and B.J. Deverall (Editors), The Dynamics of Host Defense. Academic Press, New York, NY, pp. 255–273.

Kuc, J., 1987. Plant immunization and its applicability for disease control. J. Wiley, New York, NY, pp. 255–273.

Kuc, J., Schockley, G. and Kearney, K.K., 1975. Protection of cucumber against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*. Physiol. Plant Pathol., 7: 195–199.

Lewis, A.C., 1979. Feeding preference for diseased and wilted sunflower in the grasshopper, *Melanoplus differentialis*. Ecol. Entomol., 7: 391–395.

Mattson, W.J. and Haack, R.A., 1987a. The role of drought in outbreaks of plant-eating insects. BioScience, 37(2): 110–118.

Mattson, W.J. and Haack, R.A., 1987b. The role of drought stress in provoking outbreaks of phytophagues insects. In: P. Barbosa and J.C. Schultz (Editors), Insect Outbreaks. Academic Press, San Diego, CA, pp. 365–407.

McQuate, G.T. and Connor, E.F., 1990a. Insect responses to plant water deficits. I. Effects of water deficits in soybean plants on the feeding preference of Mexican bean beetle larvae. Ecol. Entomol., 15: 419–431.

McQuate, G.T. and Connor, E.F., 1990b. Insect responses to plant water deficits. II. Effect of water deficits in soybean plants on the growth and survival of Mexican bean beetle larvae. Ecol. Entomol., 15: 433–445.

Mellors, W.K. and Propts, S.E., 1983. Effects of fertilizer level, fertility balance, and soil moisture on the interaction of two-spotted spider mites (Acari: Tetranychidae) with radish plants. Environ. Entomol., 12: 1239–1244.

Oloumi-Sadeghi, H., Helm, C.H., Kogan, M. and Schoenweiss, D.F., 1988. Effect of water stress on abundance of two-spotted spider mite on soybeans under greenhouse conditions. Entomol. Exp. Appl., 48: 85–90.

Rhoades, D.F., 1983. Herbivore population dynamics and plant chemistry. In: R.F. Denno and M.S. McClure (Editors), Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York, NY, pp. 155–220.

Sumner, L.C., Need, J.T., McNew, R.W., Doeschner, K.W., Eikenbary, R.D. and Johnson, R.C., 1983. Response of *Schizaphis graminum* (Homoptera: Aphidae) to drought stressed wheat using polyethylen glycol as a matricum. Environ. Entomol., 12: 919–922.

White, T.C.R., 1969. An index to measure wheather-induced stress of trees associated with outbreaks of psyllids in Australia. Ecology, 50: 905–909.

White, T.C.R., 1974. A hypothesis to explain outbreaks of looper caterpillars, with special reference to populations of *Selidosema suavis*, in a plantation of *Pinus radiata* in New Zealand. Oecologia (Berlin), 16: 279–301.

White, T.C.R., 1976. Weather, food and plagues of locusts. Oecologia (Berlin), 22: 119–134.

White, T.C.R., 1984. The abundance of invertebrate herbivores in relation to availability of nitrogen in stressed food plants. Oecologia (Berlin), 63: 90–105.