

## Chapter 6

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# *Verticillium biguttatum*, an Important Mycoparasite for the Control of *Rhizoctonia solani* in Potato

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## INTRODUCTION

*Rhizoctonia solani* Kühn is an important cause of damage in potato production. The damage has a quantitative as well as a qualitative aspect. Serious infection of stems reduces the productive capacity of the plant and leads to losses of yield and a reduction in starch content of the tubers (Mulder, 1974; Hülsmann, 1976; Banville, 1978). The production of sclerotia (black scurf) on the newly-formed tubers leads to a reduction in the amount of certifiable tubers. These losses are of special importance in the production of seed potatoes.

Until now, rotations in which potatoes occurred at intervals of 5 years or more were the only way to limit infection from the soil. When potato production became economically more profitable than growing other crops, the frequency of potatoes in rotations increased and consequently an increase in black scurf occurred.

Sclerotia of *R. solani* on potato tubers are often colonized by mycoparasites such as *Trichoderma* spp., *Gliocladium roseum*, other *Gliocladium* spp., *Hormiactis fimicola* and *Volutella ciliata*. By far the most frequently occurring mycoparasite is a *Verticillium* species (Jager and Velvis, 1983a,b). This fungus, *Verticillium biguttatum* Gams, is able to kill sclerotia (Velvis and Jager, 1983; Jager and Velvis, 1988) and hyphae (Jager *et al.*, 1979; van den Boogert and Jager, 1983, 1984) and consequently protects young sprouts against infection with *R. solani* (Jager and Velvis, 1984). *V. biguttatum* is the most effective of the sclerotium-inhabiting fungi in controlling *R. solani* in the field (van den Boogert and Jager, 1984).

Two sources of *Rhizoctonia* infection can be distinguished: the seed tuber and the soil. Infection on the seed tuber can easily be eliminated by chemical disinfection. Since 1982, *Rhizoctonia*-specific fungicides (tolclofos-methyl, trade mark Rizolex; pencycuron, trade mark Monceren) have been available. These fungicides can be applied to the soil and reduce

its importance as a source of infection. However, the use of high doses of fungicides in agriculture progressively threaten soil fertility (Brussaard, 1986). Therefore, other ways than the intensive use of fungicides are necessary to maintain the soil fertility and to ensure the production of seed potatoes, qualitatively as well as quantitatively.

The current review deals with the ecology of *V. biguttatum* and its applicability as a biological alternative for controlling *R. solani*.

## ECOLOGY OF *VERTICILLIUM BIGUTTATUM*

### Axenic Growth and Sporulation

*V. biguttatum* grows and sporulates on common mycological media. Normally, washed conidia of *V. biguttatum* do not germinate on purified water agar unless carbon sources are available. The carbon requirements for growth in axenic culture can be satisfied by a limited number of carbohydrates. Ammonia and glutamine appear to be the only nitrogen sources utilized by the mycoparasite in a liquid synthetic medium with glucose. The growth of *V. biguttatum* is biotin-dependent, which means that the fungus is unable to synthesize this vitamin. Mycelium is most extensive on glucose-containing media, but the largest amount of conidia per unit of mycelium is produced on galactose-containing media (van den Boogert, 1989a). Both *V. biguttatum* and *R. solani* grow readily on synthetic media, but they have different nutrient requirements. *R. solani* has a broader spectrum of utilizable substrates than *V. biguttatum* and together they can grow in a synthetic medium without vitamins.

*V. biguttatum* grows very slowly but sporulates abundantly on synthetic media. Its radial growth rate amounts to 10% of that of *R. solani*. The two fungi also have different temperature ranges for growth: 13–30°C and 0–30°C for *V. biguttatum* and *R. solani*, respectively. The pH tolerance for growth of *V. biguttatum* ranges from 3.5–5.5 in liquid culture. *R. solani* is less sensitive to pH and grows at higher pH values than *V. biguttatum*.

### Occurrence and Distribution

The presence of *V. biguttatum* on the plant or in the soil can be demonstrated by plating plant pieces or soil particles on agar plates overgrown with mycelium of *R. solani*. These “*Rhizoctonia* plates” are selective for *V. biguttatum* and constitute a convenient method for the quantitative assessment of *V. biguttatum* in the soil (van den Boogert and Gams, 1988).

The density of the mycoparasite in soil can be drastically increased by the addition of living hyphae of *R. solani* to the soil (Jager *et al.*, 1979; van den Boogert and Jager, 1983). This soil-enrichment facilitates screening for

the presence of the mycoparasite in the soil. *V. biguttatum* occurs in a diversity of soil types ranging from acid soils with a high organic matter content to neutral mineral loam soils. The fungus has been found all over the world and occurs in nearly all soil types used for potato growing (van den Boogert, unpublished). As observed in a potato field located at Haren, *V. biguttatum* mainly occurs in the upper 30 cm, but is present in deeper layers down to 60 cm (Jager and Velvis, 1989).

*V. biguttatum* is not only found on potatoes. It also occurs on roots of other crops, such as oats, wheat and sugar beet (van den Boogert, 1989b) and on the roots of various species of the natural flora such as peachwort (*Polygonum persicaria* L.), black bindweed (*P. convolvulus* L.), black nightshade (*Solanum nigrum* L.) and couch grass (*Elytrigia repens* L.) (van den Boogert, unpublished). The occurrence on plants other than potato may be attributable to the presence of *R. solani* on their roots (Jager *et al.*, 1982) or be non-specific in cases of conidia of *V. biguttatum* adhering to the roots.

## Population Dynamics

### *Survival in the soil*

Survival as dormant propagules as well as active mycelium both contribute to the longevity of many soil-borne fungi. Neither chlamydospores nor microsclerotia were observed in axenic cultures of *V. biguttatum*. Microscopic observations in soil-smear preparations revealed that the hyaline conidia of *V. biguttatum* constitute the only survival structure in the soil.

Saprophytic growth of *V. biguttatum* in soil has only been observed under circumstances of reduced microbial competition, e.g. after pasteurization (van den Boogert and Gams, 1988). Apparently, the fungus is sensitive to soil fungistasis. Fungistatic forces imposed on the conidia in soil can be overcome by addition of living mycelium of *R. solani* to the soil to which *V. biguttatum* responds by parasitizing the substrate. In this way, soils can become suppressive to the pathogen by the accumulation of *V. biguttatum* (Jager *et al.*, 1979; van den Boogert and Jager, 1983). This suppressiveness can be considerable. Approximately 90% of the sclerotia of *R. solani* added to natural soil were found to lose their viability within 3 weeks. A small fraction of the sclerotia, however, remained viable for a longer time without being infected by *V. biguttatum*. Apparently, the fraction that escaped from *V. biguttatum* contributed to the long-term survival of *R. solani* in the soil (Velvis *et al.*, 1989). The *Verticillium* population declined gradually after introduction into soil. Part of the population appeared to remain viable in soil for up to 4 years (van den Boogert and Velvis, 1989).

The chance of contact between host and mycoparasite is likely to be density-dependent. Under natural conditions their population densities

range between 0–50 and 0–10<sup>4</sup> colony-forming units (cfu) per kg of soil for *R. solani* and *V. biguttatum*, respectively. The cfu of *R. solani* obtained from field soil are mainly composed of clusters of monilioid cells or small sclerotia and rarely of hyphal material (van den Boogert and Velvis, 1989). Obviously, *R. solani* and *V. biguttatum* reside in field soil in a dormant state at critical distances from each other. In undisturbed field soil and in the absence of suitable substrates for *R. solani*, e.g. the potato plant, contact between host and mycoparasite is likely to be a rare event.

### *Survival on the plant*

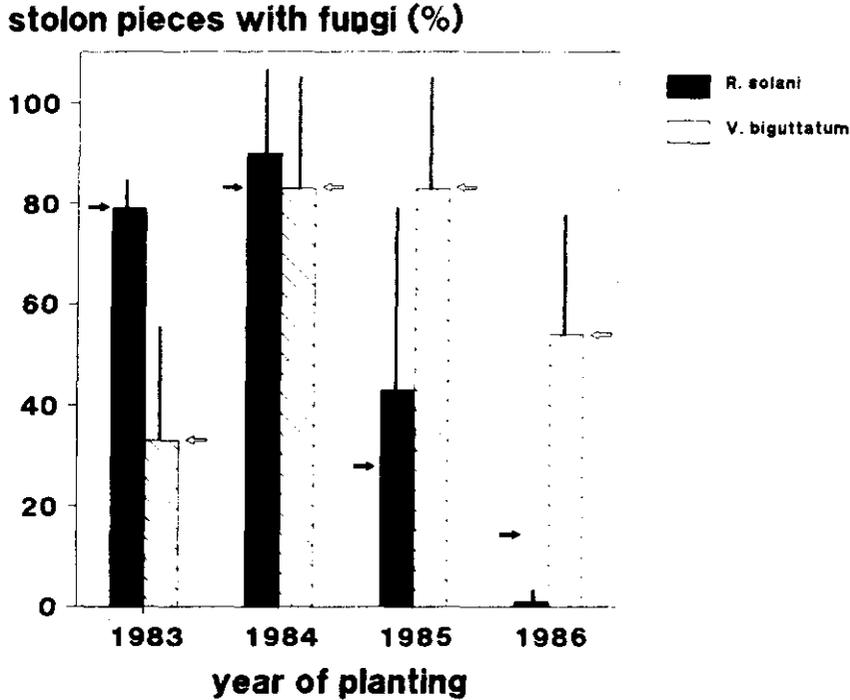
Population studies in fields continuously cropped to potato demonstrated that *V. biguttatum* only develops on *Rhizoctonia*-infected potato plants and not on *Rhizoctonia*-free plants, indicating that *V. biguttatum* is essentially a mycoparasite (van den Boogert and Velvis, 1989). The development of populations of *R. solani* and *V. biguttatum* in continuously cropped potatoes is an example of a cyclic relationship between a mycoparasite and a host fungus on which it depends for its nutrition. The population density of *V. biguttatum* cycles in concert with its host. The potato plant serves as a nutrient source and a physical base for the extension of *R. solani* through soil. Progressive growth of *V. biguttatum* eventually results in a gradual decline of *R. solani* over a 4-year period of potato cropping. The mycoparasite flourishes at first, but as food becomes limiting, its numbers decline (Figure 6.1). These alternating cycles of fungal development are not uncommon for predator-prey interactions and fit the Lotka Volterra model well (Figure 6.1);

$$\frac{dR}{dt} = 0.125 \times R - 0.002 \times R \times V;$$

$$\frac{dV}{dt} = 0.002 \times R \times V - 0.1 \times V;$$

where  $t$  = time,  $R$  = *R. solani* and  $V$  = *V. biguttatum*.

The mechanism of colonization and subsequent growth of *V. biguttatum* on subterranean plant parts were studied using PVC tubes cut longitudinally from which potato sprouts and roots could be recovered with minimum disturbance of the soil (van den Boogert, 1989b). Potato sprouts, 1 cm in length, were treated as follows: A, only the bottom 5 mm of the sprout dipped into a conidial suspension of *V. biguttatum*; B, as A plus inoculation of the bottom part of the sprout with one sclerotium of *R. solani*; C, the whole sprout dipped in the conidial suspension. After 28 days' incubation, developing sprouts and roots were examined for the presence of the two fungi at various distances from the original inoculum. The results clearly indicate that *V. biguttatum* is a poor colonizer of sprouts and the root system (Figure 6.2A). *V. biguttatum* is spread by myco-



*Figure 6.1* Population dynamics of *R. solani* and *V. biguttatum* on the stolon system of potato during 4 successive years of potato cropping; bars indicate standard deviations; arrows indicate simulated data according to the Lotka-Volterra model.

parasitic growth on developing *R. solani* and also through growth of the plant (Figures 6.2B and 6.2C). The sprout provides the fungus with a unique opportunity to be transported through soil as the plant grows and expands.

Microscopic examination of the soil-root interface confirmed the obligate mycoparasitic nature of *V. biguttatum*: germination and subsequent growth only occur in the presence of *R. solani*. Although with reduced microbial activity in the soil, e.g. after pasteurization, *V. biguttatum* conidia germinate in large numbers and even in the absence of a plant. Hence *V. biguttatum* is an ecologically obligate mycoparasite which behaves as an obligate mycoparasite in nature (van den Boogert, 1989b).

During tuber maturation at the end of the growing season, which is approximately 3 weeks, the subterranean population of *V. biguttatum* declines. The increase in its population that occurred during plant growth is nearly nullified in that period (van den Boogert and Velvis, 1989). It is possible that this decrease in population density explains a weak correlation between low *Rhizoctonia* infestation of the stolon system shortly before tuber maturation and the large number of sclerotia on the matured tubers

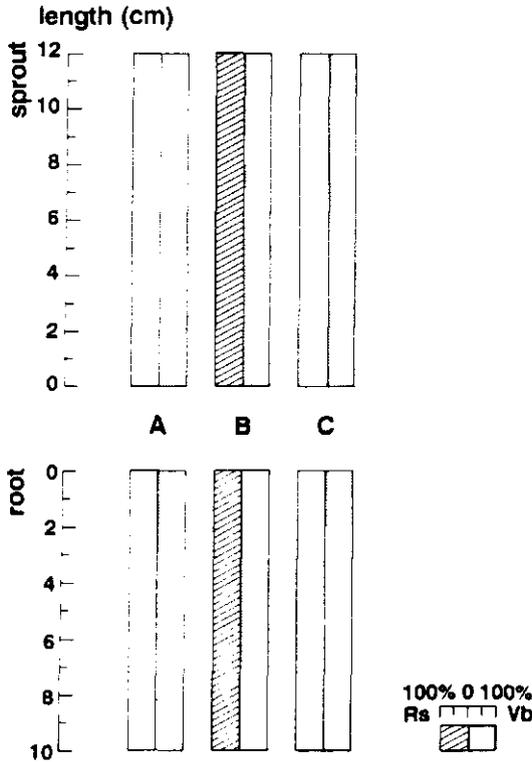


Figure 6.2 Distribution patterns of *R. solani* (Rs) and *V. biguttatum* (Vb) on potato sprouts and roots, expressed as percentage of 1-cm segments (N = 10) infected, 28 days after inoculation of the germ sprout: A, with *V. biguttatum* on the bottom half; B, as A plus a sclerotium of *R. solani*; C, *V. biguttatum* over the entire germ sprout.

at harvest. Recent experiments indicate the involvement of a predaceous fauna (van den Boogert, unpublished). Curl (1988) suggested that mycophagous protozoa, nematodes and micro-arthropods interact with soil fungi. Such organisms could also be responsible for the reduction of *V. biguttatum* and/or *R. solani* in the soil.

The pathosystem of the potato probably contains at least three interaction levels, each with a negative feedback: 1. between the potato plant and *R. solani*; 2. between *R. solani* and *V. biguttatum*; 3. between *V. biguttatum* and mycophagous fauna. Generally, very little is known about the dynamics of fungal-fungal interactions in relation to mycophagous soil fauna and more research is needed in this interesting area.

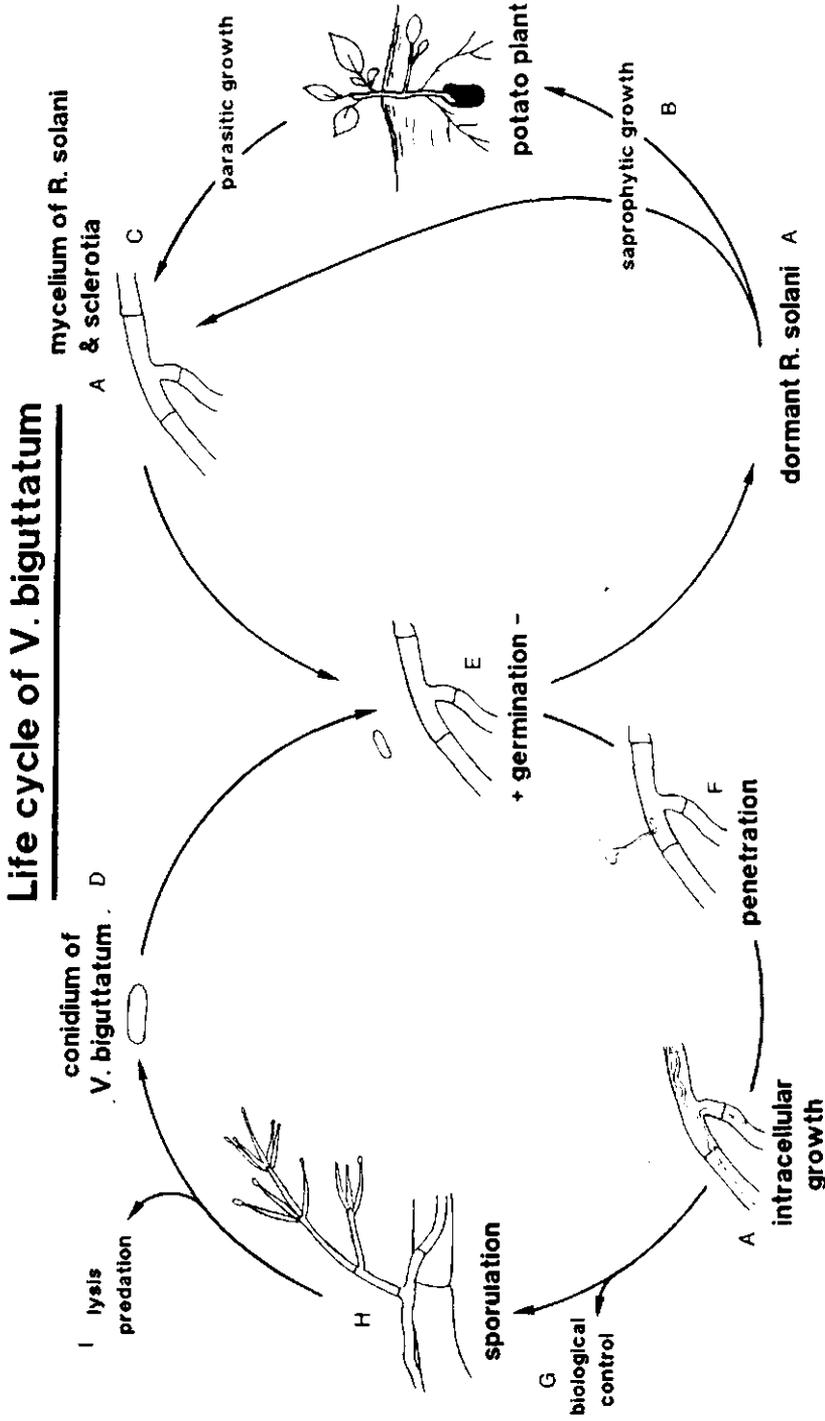


Figure 6.3 A schematic representation of the life cycle of *V. biguttatum* in relation to the parasitic and saprophytic growth of its host fungus, *R. solani*.

## Host Specificity

Many mycoparasitic fungi are not very host specific. The host range of *V. biguttatum* was determined by studying interactions with 81 different fungi on water agar plates. Interactions were diverse and varied from penetration of host hyphae, through germination without penetration of the host to no response at all. With most of the fungi tested, germ tube development of *V. biguttatum* was poor and germ tubes were distorted. With *R. solani* the germ tubes of *V. biguttatum* developed readily and were orientated towards the host, indicating the release of attracting substances by the host fungus. Particular fungi may release inhibitory substances which prevent germination or distort germ tube development of *V. biguttatum*. Hyphal contact with, and penetration of, the host cell wall were regularly observed with certain fungal species. Subsequent intracellular growth was found to occur most frequently in *R. solani*, binucleate *Rhizoctonia* and a few *Ceratobasidium* species. The different strains of *R. solani*, representing the different anastomosis groups, and *Sclerotinia sclerotiorum* enabled *V. biguttatum* to complete its life cycle by the production of a new generation of conidia. In contrast to *R. solani*, *S. sclerotiorum* allowed *V. biguttatum* to sporulate only on its mycelium and not on its sclerotia. Of the fungi tested, *R. solani* appears to be the most suitable host fungus for the mycoparasitic *V. biguttatum* (van den Boogert *et al.*, 1989).

## Life Cycle

The life cycle of *V. biguttatum* is schematically represented in Figure 6.3 and ultrastructural details of the mycoparasitic interaction are given in Figure 6.4. Pathogenic *R. solani* can survive long periods of adverse conditions in the soil by producing sclerotia and clusters of monilioid cells. When environmental conditions become favourable in combination with nutrient supply, dormancy is broken (Figure 6.3A) and *R. solani* grows saprophytically (Figure 6.3B) through the soil. Upon contact with a potato plant it produces an extensive mycelium and lives parasitically (Figure 6.3C). Both during saprophytic and parasitic growth *R. solani* may make contact with conidia of *V. biguttatum* in the soil (Figure 6.3D). Attraction of *V. biguttatum* may be in response to diffusion of attractants via the water phase. Then the conidium germinates (Figure 6.3E) and grows towards the host hypha by chemotrophy. Whether a specific recognition mechanism is involved at this stage is not yet known. After physical contact with the hyphal cell, the tip of the germ tube penetrates the cell wall (Figure 6.3F and 6.4B,C) and trophic hyphae further infect the cell (Figure 6.4A). Adjacent cells are soon invaded by the mycoparasite passing through the dolipores (Figure 6.4D) or passing through the cross walls (Figure 6.4E). The mycelium of *R. solani* is killed by expanding intra-

cellular growth of the mycoparasite (Figure 6.3G). At a later stage, conidiophores appear on the dead host and produce a new generation of conidia (Figure 6.3H). The new conidia may suffer lysis or predation (Figure 6.3I), and only a few of them will have a chance to meet *R. solani* again (Figure 6.3J).

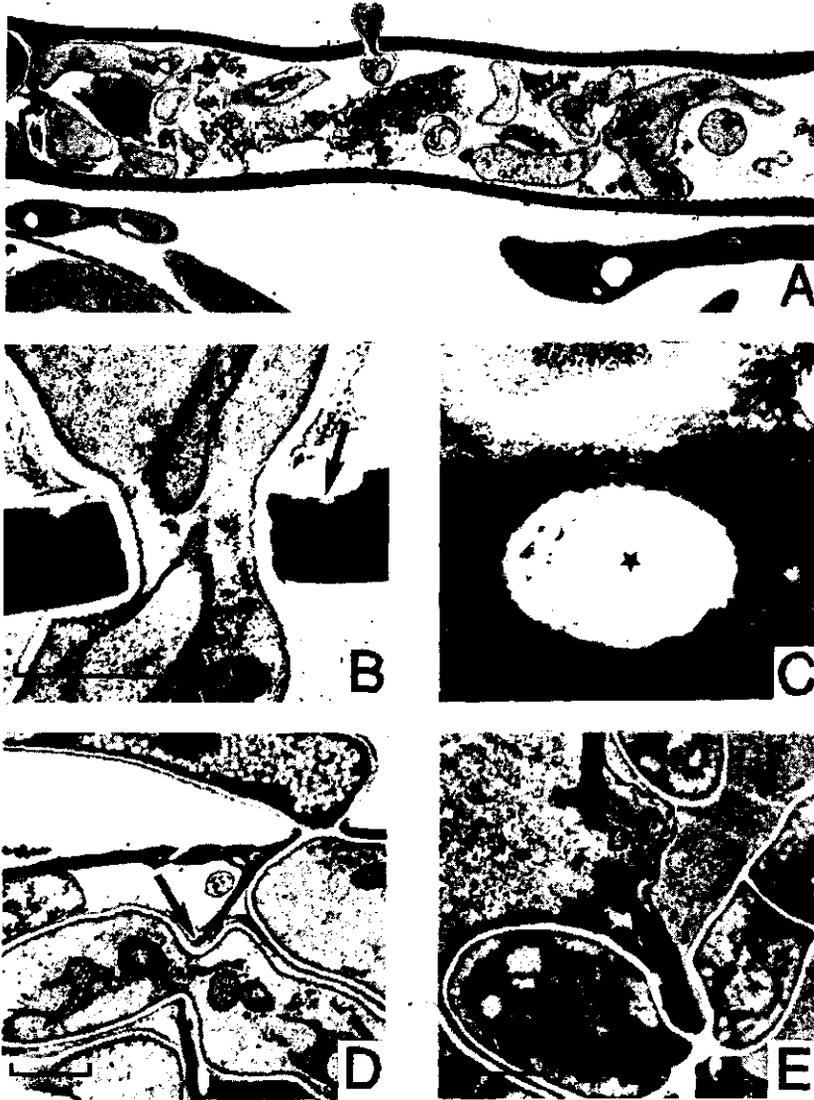


Figure 6.4 (a) Detail of a cell of *R. solani* infected with *V. biguttatum*; (b) the site of infection showing lysis of the cell wall of *R. solani* (arrow); (c) a circular penetration spot in tangential section (asterisk); (d) infected cells of *R. solani*, showing intracellular growth of the mycoparasite through the dolipore (arrow) and (e) through the cross wall (D = dolipore). (Van den Boogert *et al.*, 1989.)

## CONTROL OF *R. SOLANI* BY *V. BIGUTTATUM*

### Biological Control

The suitability of *V. biguttatum* as a control agent of *R. solani* was evaluated in a series of experiments under prevailing climatic conditions in which conidia of *V. biguttatum* were applied as a seed tuber inoculant (Jager and Velvis, 1985, 1986). The procedures for mass production of the inoculant have been described by van den Boogert and Jager (1984) and Jager and Velvis (1985; 1986). Daylight-sprouted seed tubers (cvs Bintje, Prominent and Astarte) were either dipped in or sprayed with a suspension of  $1-3 \times 10^6$  conidia/ml. They were then grown in ridges using the top 10 cm of soil and the crop managed according to farmers' practice. At the end of the growing season the production of sclerotia on newly-formed tubers was rated and expressed as a sclerotium index (SI): tubers from 10 plants were classified according to a five-step scale ranging from visually clean to heavily contaminated with sclerotia, and the SI calculated on a weight basis (Jager and Velvis, 1985). Treatments were carried out using five to ten replications. Forty-five experimental plots were planted between 1983-1986. The soil was either a sandy soil or a sandy loam soil (Figure 6.5).

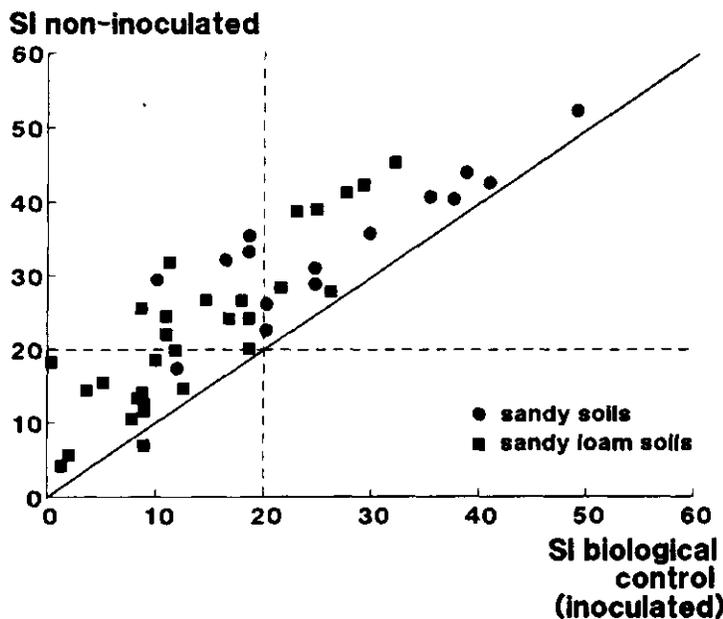


Figure 6.5 Effect of seed tuber inoculation (biological control) with *V. biguttatum* on the sclerotium index (SI) compared with a non-inoculated control. The dotted lines represent the NAK (see text) limits (SI < 20) of certifiable yield.

The biological control of *R. solani* is presented as a ratio of the SI of the non-inoculated and the inoculated treatment. Inoculation of seed tubers with *V. biguttatum* significantly reduced the production of sclerotia on the newly-formed tubers and the average SI reduction amounted to 25.9% ( $P=0.01$ ) on the sandy soils and to 40.0% ( $P=0.01$ ) on the sandy loam soils. Regression analysis was used to analyze the data. A *Rhizoctonia* SI of 20 is the maximum level of infection accepted by the General Dutch Inspection Service of Seeds (NAK) for certified seed potatoes. With inoculation, 32 out of 45 experimental fields met that requirement, but only 17 fields did so without the inoculation.

Another practical application of *V. biguttatum* is the biological disinfection of the potato tubers by spraying them with the conidial suspension shortly after harvesting. As a consequence, the viability of the *Rhizoctonia* sclerotia is strongly reduced during storage, although the dead sclerotia remain attached to the tubers (Table 6.1). Biological seed tuber disinfection is only successful if direct contact between conidium and sclerotium is obtained, the temperature during storage is at least 15°C, and the humidity of the air around the tubers is at least 99% (Jager and Velvis, 1988).

Table 6.1 Percentage of dead sclerotia of *R. solani* on potato tubers with (+) and without (-) inoculum of *V. biguttatum* after incubation for different times and at different temperatures (Jager and Velvis, 1988).

Incubation temperature (°C)	<i>V. biguttatum</i>	Dead sclerotia (%) after			Sclerotium index after 47 days
		17 days	31 days	45 days	
14	-	5	8	10	61
	+	5	20	22	56
17	-	22	20	27	59
	+	79	86	95	58
20	-	12	20	38	56
	+	67	85	89	59

### Integrated Control

Treatment of the soil with Rizolex or Monceren at  $1/2$  and  $1/10$  of the recommended dose (20 kg/ha) allows good development of *V. biguttatum* on potato sprouts (Jager, 1987). These results prompted field experiments to compare chemical and integrated control of *R. solani*.

The fungicides Monceren or Rizolex were suspended in water and sprayed on the surface of the soil just before the ridges were made. Fungicides were added to the soil at  $1/2$ ,  $1/4$  and  $1/8$  the recommended dose. Integrated control consisted of soil treatment with one of the fungicides in

combination with seed tuber treatment with *V. biguttatum* (Jager *et al.*, 1989). The tubers of ten plants were rated for sclerotia and a *Rhizoctonia* SI was calculated. Treatments were replicated ten times.

The effectiveness of integrated control is presented as a ratio of the SI of integrated and chemical treatments obtained at 20 experimental fields (Figure 6.6). Integrated control with *V. biguttatum* is more effective in reducing the amount of sclerotia than the chemical treatment alone. The average SI reduction by *V. biguttatum* amounted to 44.3% ( $P=0.01$ ) for sandy soils and 43.7% (NS at  $P=0.05$ ) for sandy loam soils. With integrated control of *R. solani*, 18 plots out of 20 yielded potatoes certifiable by NAK standards (SI < 20). With fungicide treatment alone only 13 out of 20 plots received such a certification. This clearly indicates that the combination of seed tuber treatment with *V. biguttatum* and a soil treatment with a low dose of a fungicide gives better results than the soil treatment with fungicides alone.

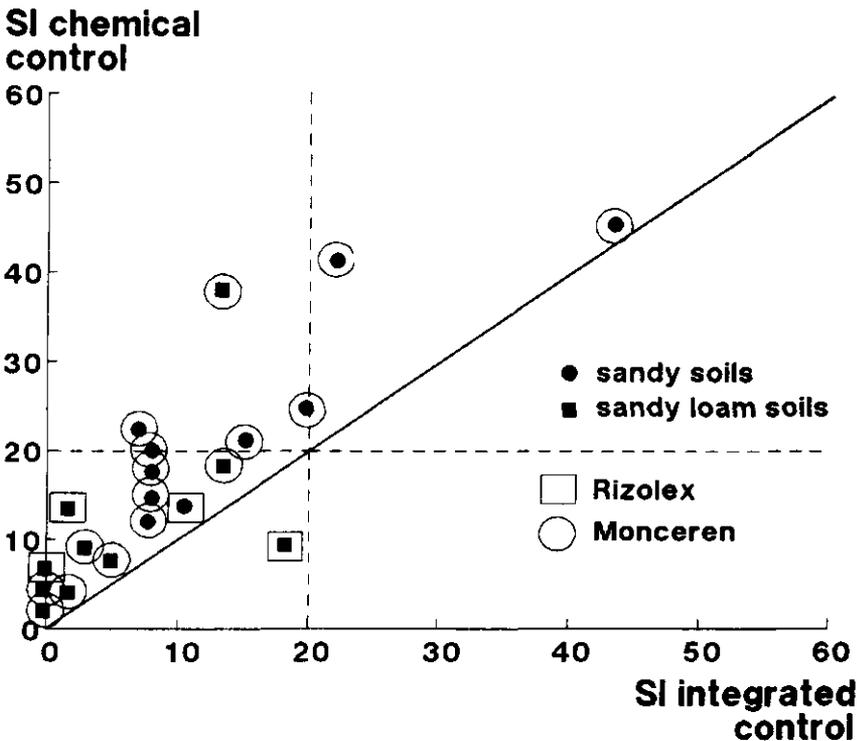


Figure 6.6 Effect of the integration of biological control (seed tuber inoculation with *V. biguttatum*) and chemical control (soil application of fungicides) on the sclerotium index (SI) compared with the effect of chemical control alone. The dotted lines represent the NAK (see text) limits (SI < 20) of certifiable yield.

## CONCLUDING REMARKS

For its natural development, *V. biguttatum* is completely dependent on *R. solani*, which in turn depends on the potato plant as a substrate (see life cycle in Figure 6.3). The *V. biguttatum*-*R. solani* relationship is a good example of a cyclical relationship: the mycoparasite depends upon the host for substrate and its population density fluctuates with that of its host. In axenic culture the mycoparasite grows readily without its host and is therefore regarded as an ecologically obligate mycoparasite of *R. solani*. Ecologically *V. biguttatum* is more restricted than its host. This apparently enables *R. solani* to escape from *V. biguttatum* when circumstances are less suitable for the mycoparasite.

The modes of action of Monceren and Rizolex are not well understood. The fungicidal action of Monceren is extremely selective, even within the *Rhizoctonia solani* group (Kuck *et al.*, 1988). We have some circumstantial evidence that the pathogenesis of *R. solani* is negatively affected by Monceren (G. Jager, unpublished work).

Integrated control of *R. solani* is more advantageous than chemical or biological control because:

1. less fungicide is needed;
2. resistance of *R. solani* to the fungicide is less likely to develop and resistance to *V. biguttatum* does not build up;
3. the chemical component of the integrated system is already active before the soil temperature required for activity of the biological component is reached.

Our results are reason to be optimistic about successful application of biological and integrated control of *R. solani* in agriculture.

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