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Stem application of metalaxyl for the protection of *Vitis vinifera* L. ('Riesling') leaves and grapes against downy mildew (*Plasmopara viticola*)

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Summary

The direct application of plant protective agents into tree xylem is an ecologically sound method of plant protection, which has already been used for trees in urban environements. In order to make this system viable for grapevines (*Vitis*), the effectiveness of the protective system must be ensured.

In the first experiment, the effect of the fungicide agents dimethomorph, fosetyl-al, iprovalicarb and metalaxyl applied to the stem of field-grown grapevines was investigated. As a result, injection experiments using non-formulated metalaxyl showed the desired protective effects against downy mildew (*Plasmopora viticola*). In the second experiment, non-formulated metalaxyl was applied several times in succession over a period of several weeks to the xylem of test grapevines using an injection system, affixed long-term to the vine stems. Grapevines sprayed with formulated metalaxyl (Fonganil Gold®) enabled the methods to be compared. The efficiency factors with regard to the control of downy mildew in leaves and grapes were very similar in both approaches, with values of practical relevance of over 70 %.

Key words: Downy mildew, fungicide, grapevine, stem injection, stem application, viticulture, xylem.

Introduction

Unlike with farmland cultures, the main emphasis of applying plant protective agents in viticulture is not primarily weed control, but rather to combat pests and fungal diseases. Depending on the course and intensity of the epidemic, several treatments of pesticides and fungicides are usually required per vegetation period. Compared with pesticide application in farmland cultures, the following differences arise:

Drift is more marked in viticulture than in farmland cultures due to the height of plants and the resulting spraying technique. State-of-the-art technology uses recycling devices to reduce driftage losses in flat areas (Hillebrand *et al.* 1998), but in practice these are not yet very widespread.

In steep areas fungicides may be applied using helicopters, which can result in a higher contamination of non-target areas (Mohr and Holz 1995). In these regions at least, pesticides must be applied using knapsack sprayers (Mohr and Holz 1995, Hillebrand *et al.* 1998). Applying products by tractor is both labour- and cost-intensive, since the rows of grapevines must be driven along individually. Since the preparations need to be applied several times throughout a single vegetation period, expenditure is multiplied accordingly.

The entry of plant protective agents used in viticulture into the environment cannot be prevented using conventional methods of application.

An environmentally neutral supply of plant protective agents to the grapevines can only be guaranteed in a closed application system with a direct connection to the vine organism (Düker *et al.* 2006). Not only would stem application of the spray solution to the grapevine lead to no loss, but also the total input of the solution per hectare would be reduced.

A number of articles on inserting substances directly into the vascular tracts of certain plants have been published in the literature since the mid-1970s. These experiments usually used single injections into individual avenue trees (Prasad 1974), economically cultivated plantation (Clifford *et al.* 1987, Bayer 2000) and forestry trees (Mc Cain 1980, Phair and Ellmore 1984, Lanier 1987) or natural monuments (Prasad 1974). In order to make the trunk injection method viable for grapevines, the objectives of the experiments presented here were (a) to investigate the effect of some xylem-applied active ingredients of fungicides against downy mildew, which is highly economically relevant; (b) to evaluate the use of non-formulated metalaxyl over a period of several weeks by means of stem application compared to spraying with Fonganil Gold®.

Material and Methods

To achieve the aims of this study, the following experiments were conducted: Single xylem injections of some active ingredients of fungicides (dimethomorph, fosetyl-al, iprovalicarb and metalaxyl) to test the effects against downy mildew. The repeated xylem application of non-formulated metalaxyl with a prototypal long-term injection system (Düker *et al.* 2006) to evaluate the longer-term effects with regard to controlling downy mildew in the leaves and grapes of treated grapevines. At the same time, formulated metalaxyl (Fonganil Gold®) was also repeatedly sprayed to enable the practical comparison of both methods.

Material: Downy mildew (*Plasmopora viticola*) was used as the test organism. Tests were performed on 20-year-old field-grown 'Riesling' grapevines. General

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plant protection was discontinued six weeks before tests commenced. Only the plants in the second experiment were sprayed twice with Aliette Flash® (800 $g \cdot l^{-1}$ fosetyl-al) as a precaution. At this time, the plants were not affected with downy mildew because of the low disease pressure, probably due to the low temperatures and rainfall in the months of April and Mai (Tab. 1).

Table 1

Meteorological data of the Palatinate (Germany). Source: Dienstleistungszentrum Ländlicher Raum (DLR) Rheinpfalz, Breitenweg 71, D- 67435 Neustadt, Germany

2006	Average temperatures	Rainfall
	Monthly average (°C)	Monthly totals (mm)
January	-0,4	15
February	1,8	26
March	4,5	45
April	10,6	36
May	15,6	54
June	19,3	99
July	24,6	55
August	17,1	152
September	18,3	91
October	13,5	100
November	8,3	21
December	4,9	25

Since the potential occurrence of powdery mildew (*Unicula necator*) and grey mould (*Botrytis cinerea*) on the test plants outdoors would have negatively influenced the growth of downy mildew, the field-grown grapevines were treated with the fungicides Prosper® (500 $g \cdot l^{-1}$ spiroxamine), Vento® (200 $g \cdot l^{-1}$ quinoxyfen and 60 $g \cdot l^{-1}$ fenarimol), Ronilan® (500 $g \cdot l^{-1}$ vinclozolin), Fortress 250® (500 $g \cdot l^{-1}$ quinoxyfen) and Topas® (100 $g \cdot l^{-1}$ penconazol) several times in the period leading up to the commencement of the experiment. These fungicides were selected because they did not possess any additional effects against downy mildew.

Methods - Injection: In the first experiment, the stem applications were carried out using two ChemJet® tree injectors per grapevine. The injectors were purchased from CHEMJET® Trading, Pty. Ltd., P.O. Box 318, Caboolture QLD 4510, AUSTRALIA. A ChemJet® is a device containing a coil spring, which resembles an application syringe. By dipping the tip into the fluid to be applied and simultaneously drawing back the piston against the power of a coil spring, the fluid is drawn up into the chamber of the injector. The uptake volume is 20 ml. By turning the piston clock-wise, it can be locked into the appropriate apparatus. In order to mount the injectors, a 4 mm hole is drilled into the narrow side of each grapevine trunk. The tree injectors are then affixed into the drilled holes by rotation. Once the catches on the ChemJets® are released, the actual application of the fluid commences. Stem injections with the fungicide agents dimethomorph (1.29 $g \cdot l^{-1}$ H_2O), fosetyl-al (8.93 $g \cdot l^{-1}$ H_2O), iprovalicarb (0.97 $g \cdot l^{-1}$ H_2O) and metalaxyl (0.53 $g \cdot l^{-1}$ H_2O) were performed on 6 field-grown grapevines on 23 may 2006. 51.4 mg dimethomorph, 357.0 mg fosetyl-al, 38.6 mg iprovalicarb and 21.0 mg metalaxyl were injected per grapevine. These amounts corresponded to the quantities applied in practice by sprinkling and spraying per grapevine for the respective stage of development. Water was applied to six further grapevines, which served as control plants.

In the second experiment, stem injections were applied using a prototypal stationary injection system, intended for use over several years (DÜKER *et al.* 2006), which was already affixed to eight field-grown grapevines one year prior to the experiment. In this system, water from a stainless steel tank is initially conveyed via an ultraviolet lamp, for sterilisation purposes, to a membrane pump, which drives the system. The water is subsequently pushed through tubing to the grapevines. Regularly spaced, individual inlet pipes lead from the main inlet pipe, which was laid along the lower wire frame in the grapevine row, to the grapevines. Three series connected injector units, each consisting of a needle and its attachment, are affixed to each of these inlet pipes. The needles, which are pressed into the vine stem during assembly using special pliers, facilitate direct access to the grapevine vessels. The pressure of the system is kept constant at 4 bar by a valve. Plant protection takes place according to requirements via a dosage loop, which is connected upstream of the main inlet pipe. To this aim, the fluid to be applied is initially drawn up using a syringe and is introduced into the dosage loop via a valve. The stem injections with non-formulated metalaxyl (0.59 $g \cdot l^{-1}$ H_2O) took place six times successively at ten- to twelve-day intervals, commencing on 23 June 2006. Each time, 270 ml of the application solution was introduced into the prototypal injection system via the dosage loop. Under the proviso that the application solution is distributed evenly to each inlet pipe, each grapevine was therefore injected with 20 mg metalaxyl per application. Sixteen further untreated grapevines acted as control plants. Furthermore, in order to enable the direct comparison of the stem injection method and the other conventional methods of application by means of spraying and sprinkling, 27 grapevines were treated with formulated metalaxyl (Fonganil Gold®). The plants were sprayed six times successively, at the same time as the stem injections. The quantity applied was adjusted to the plants' respective stage of development, and amounted to 17.5 mg metalaxyl/grapevine for the first spray, 24.5 mg metalaxyl/grapevine for the second and 28.0 mg metalaxyl/grapevine for all subsequent sprays.

Inoculation: A metalaxyl sensitive strain of downy mildew (*Plasmopora viticola*) was used as the test organism. The suspension produced for inoculation contained 50,000 $sporangia \cdot ml^{-1}$. In the first experiment, two shoots with several day-old leaves were inoculated per grapevine on 1 June 2006, 9 d after stem application of the fungicide agents. At that time, the plants were not yet in blossom (Tab. 2). In the second experiment, one shoot and cluster was inoculated per grapevine four and eight days, respectively, after the first application of metalaxyl, which was carried out on 23 June 2006. The inoculations were performed on several day-old leaves or in the clusters located in the shedding blossom.

Table 2

Phaenological development of grapevines in Neustadt (Rhineland-Palatinate, Germany). Source: see Tab. 1

Grapevine phenology	2006
Bud swelling	19. April
Shoot	26. April
Commencement of blossing	13. June
Pea-sized berries	05. July
Commencement of maturity	17. August

The shoots and clusters were inoculated in the evening to prevent the sporangia from being damaged by sun-rays. For this, a dash of the suspension was applied to the marked shoots and clusters using a wash bottle. Immediately after inoculation, a plastic bag, moistened on the inside with distilled water, was placed over each shoot and cluster and the bag was fastened to the shoot's and cluster's stalk using a binding wire. The humidity achieved under the bags was intended to guarantee that the sporangia germinate overnight. The bags were collected the next morning to prevent the germinating sporangia from becoming damaged by overheating. At this point, the leaves and clusters were still dripping wet.

Rating and interpretation: In the first experiment, a one-off estimation of the inoculated leaves of control and test variants took place three weeks after inoculation with downy mildew.

In the second experiment, infestation estimations were performed on a weekly basis on 400 non-inoculated leaves and 200 non-inoculated grapes, respectively, per variant. An exception to this was the stem application variants, since only eight grapevines were connected to the long-term injection system. In this case, estimations were performed on 200 non-inoculated leaves and 100 non-inoculated grapes, respectively. The infestation estimations of the leaves and grapes commenced on July 20, 2006 and August 2, respectively.

The leaves and grapes to be estimated were classified into groups according to the percentage of affected leaf area and percentage of infected berries, respectively (0 %, up to 5 %, up to 10 %, up to 25 %, up to 50 %, up to 75 % and up to 100 %). The infestation frequency of the individual variants is yielded from the following formula:

$$IF\ [\%] = ((A + B + C \ldots / N) \times 100),$$

whereby the free variable parameters A, B, C, … represent the number of leaves and grapes that were classified to the respective estimation groups, and N denotes the total number of leaves and grapes of each respective variant. The intensity of infestation of the individual variants was calculated using the following formula:

$$IOI\ [\%] = (mc_1 \times A) + (mc_2 \times B) + (mc_3 \times C) \ldots / N,$$

where mc represents the mid-point of class, the free variable parameters A, B, C, … are the number of leaves and grapes that could be classified to the respective mid-point of class, and N represents the total number of leaves and grapes of each respective variant. The efficiency factor of the agents and/or application methods was derived using the following formula:

$$EF\ [\%] = ((IOI_{CP} - IOI_T) / IOI_{CP}) \times 100,$$

where IOI_{CP} is the intensity of infestation of the control plants and IOI_T is the intensity of infestation of the treated grapevines. The data collected in the first experiment were subjected to Tuckey's test (Alpha ≤ 0.05). The values gained from second experiment underwent a simple linear regression.

Results and Discussion

Selection of suitable fungicide agents: Fig. 1 A illustrates the resulting infestation fre-

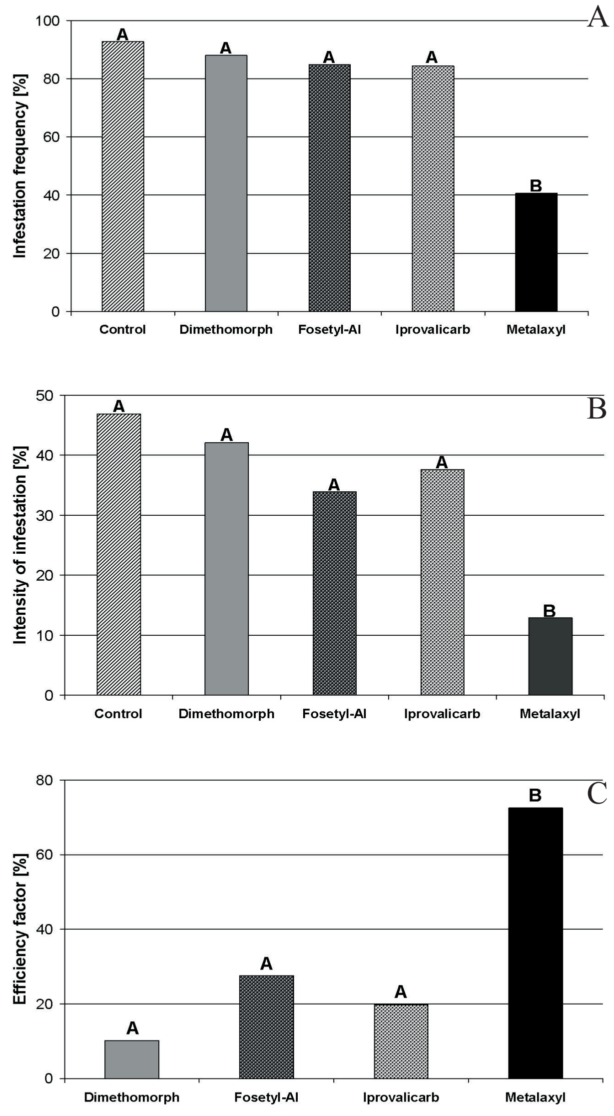

Fig. 1: Results of individual injections of fungicide agents (dimethomnorph, fosetyl-al, iprovalicarb, metalaxyl) into the xylem of field-grown grapevines. Injections were carried out using two ChemJet® injectors per grapevine. The values resulted from 6 repetitions (control plants), 5 repetitions (fosetyl-al) and 4 repetitions (dimethomorph, iprovalicarb, metalaxyl), respectively. Capital letters above the columns represent the statistical difference Alpha ≤ 0.05 within each test variant. **A**) Infestation frequency of downy mildew on the leaves of test variants and control plants. **B**) Intensity of infestation with downy mildew on the leaves of test variants and control plants. **C**) Efficiency factors of the test variants.

quency of downy mildew on the leaves of each respective variant from the first experiment. As expected, the value of the control plants (92.8 %) was highest. Although the infestation frequencies of the test variants with dimethomorph (88.0 %), fosetyl-al (84.8 %) and iprovalicarb (84.4 %) revealed lower values, they did not differ significantly from the value gained by the control plants. The by far lowest infestation frequency was determined with the variant in which metalaxyl was applied (40.7 %).

Fig. 1 B presents the intensity of infestation of the individual variants. The value of the control plants was 46.9 %. The intensity of infestation of the test variants with dimethomorph (42.1 %), iprovalicarb (37.6 %) and fosetyl-al (33.9 %) were lower than that of the control plants, but did not differ greatly from them. Once again, the test variant with metalaxyl (12.9 %) generated the lowest value in this case.

The efficiency factor of the stem injected fungicide agents are shown in Fig. 1 C. Metalaxyl achieved a value of 72.5 %, which is of practical relevance. Fosetyl-al (27.6 %), iprovalicarb (19.8 %) and dimethomorph (10.2 %) achieved only low efficiency factors.

The result of this experiment was therefore merely that one fungicide agent, applied by means of stem injection, achieved practically relevant protection against downy mildew, even without being combined with other preparations. As with the conventional practice of spraying and sprinkling, an increase of efficiency can probably only be achieved by combining different agents. In this context, for example, the combination of metalaxyl, which inhibits protein synthesis by disturbing the nucleic acid synthesis of *Plasmopora viticola*, with fosetyl-al, which tends to prevent spore germination, are conceivable. Adequate anti-resistance management by means of stem injection, however, is currently unrealistic because the required number of suitable substances with accordingly high efficiency factors is lacking.

The effect of stem injected metalaxyl: The resulting infestation frequency of downy mildew on the leaves of each variant from the second experiment is presented in Fig. 2 A. Even at the beginning of the estimations, the values of the stem injected variants (22.0 %) and the spray-applied variants (18.0 %) were below that of the control plants (30.5 %). The infestation frequency of the control plants increased continuously throughout the estimation period, and reached 43.5 % towards the end of the test. Unlike with the control plants, the values of the two test variants were subject to fluctuations. However at the end of the tests, the infestation frequency of the injected variant was 25.5 % and that of the sprayed variant 12.3 %; both cases were therefore lower than the value of the control plants. If the linear regression is included, the infestation frequency of the sprayed-applied variant even revealed a decreasing tendency during the estimation period. The infestation frequency of the stem injected variant, on the other hand, tended to increase slightly.

Fig. 2 B presents the determined intensity of infestation on the leaves of control and test variants. Even at the beginning of the tests, the control plants (5.3 %) showed a higher intensity of infestation than the two test variants (3.1 % for stem injection and 1.8 % for application by spraying). During the estimation period, the values of all variants were subject to fluctuations. However, the determined intensity of infestation of the control plants was always considerably higher than the values of the two test variants. Moreover, the intensity of infestation of the control variant also revealed the highest increase with regard

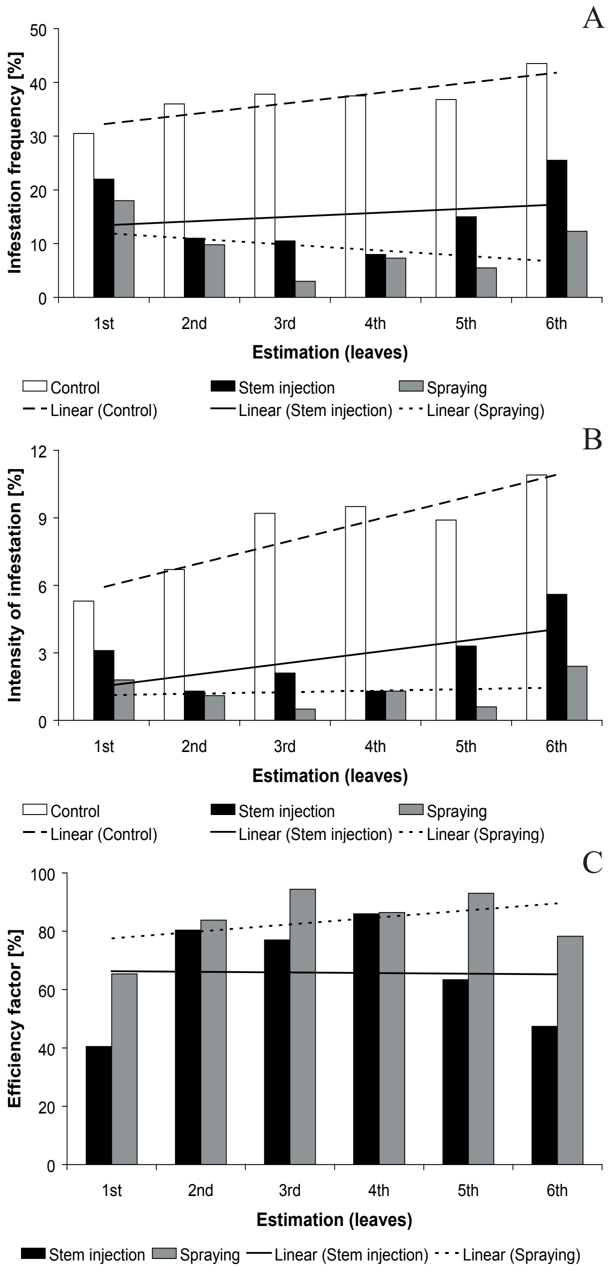

Fig. 2: Results of the repeated xylem injection of non-formulated metalaxyl with a prototypal long-term injection system compared with the simultaneous spraying of the formulated product (Fonganil Gold®). The values were determined from the estimations of 400 (control plants, sprayed application) and 200 leaves (stem injection) per variant. The regression lines were generated using Microsoft Office Excel 2003. **A**) Infestation frequency of downy mildew on the leaves of test variants and control plants. **B**) Intensity of infestation with downy mildew on the leaves of test variants and control plants. **C**) Efficiency factors of the test variants.

to the linear regression. The determined efficiency factor concerning the two test variants is presented in Fig. 2 C. Even on the second estimation date, both variants revealed an efficiency factor of over 80 %. The highest value was achieved by the spray-applied variant (94.4 %) on the third estimation date and by the stem injection variant (86.0 %) on the fourth estimation date. Since the use of plant protection was discontinued two weeks prior to the end of the tests, however, the efficiency factor of the stem injection variant decreased again towards the end. When including the linear regression, the efficiency factor of the spray-applied variant revealed an tendency to increase. The efficiency factor of the stem-applied variant, on the other hand, tended to decrease slightly.

The protective effect against downy mildew on leaves, achieved by the stem injection of non-formulated metalaxyl, is therefore comparable with the effect of the sprayed formulated product (Fonganil Gold®) throughout the phase of regular plant protection applications. However, the effect of stem-applied Metalaxyl rapidly weakened after plant protection was stopped.

Fig. 3 A presents the resulting infestation frequency of downy mildew on grapes of the respective variants from the second experiment. Even at the beginning of the tests, the value of the control plants (50.1 %) was very high. The infestation frequency of the two test variants (21.0 % for application by spraying and 13.5 % for stem injection), however, was considerably lower. During the tests, the infestation frequency of all variants increased. Towards the end of the estimations, values of 66.0 % (control plants), 61.0 % (stem injection) and 37.5 % (spray application) were generated. This development was also reflected in the portrayed regression lines: The infestation frequency of the stem-applied variant revealed the greatest tendency to increase.

Fig. 3 B illustrates the respective intensity of infestation of the control and test variants. Even at the outset of the estimations, the value of the control plants was 15.3 %. The intensity of infestation of both test variants was considerably below this amount (4.4 % for application by spraying and 3.0 % for stem injection). This tendency remained for almost the whole duration of the tests. Towards the end, however, the intensity of infestation increased in all variants because plant protection was discontinued three weeks prior to the end of the estimation.

The efficiency factor of each test approach is illustrated in Fig. 3 C. Even at the beginning of the tests, high values were obtained for both the stem injection variant (80.7 %) and the spray application variant (71.6 %). This tendency was observed up to the final application of the fungicide agent. Once plant protection was discontinued, however, the protective effect decreased in both variants. The efficiency factor of both variants therefore revealed a tendency to decrease, when including the linear regression.

The protective effect, achieved by means of the stem injection of non-formulated metalaxyl with regard to downy mildew in grapes, which conformed with the effect of the sprayed formulated product (Fonganil Gold®), thus confirms the suitability of the method. Although Fonganil Gold® is not a registered fungicide to control downy mildew in viticulture and it is not as efficient as registered fungicides, this result is nevertheless an indication that the stem application of suitable agents can principally be used to control *Plasmopora viticola*.

E c o l o g y a n d e c o n o m i c s o f t h e m e t h o d : Unlike with conventional spraying methods, stem application does not lead to emissions into the envi-

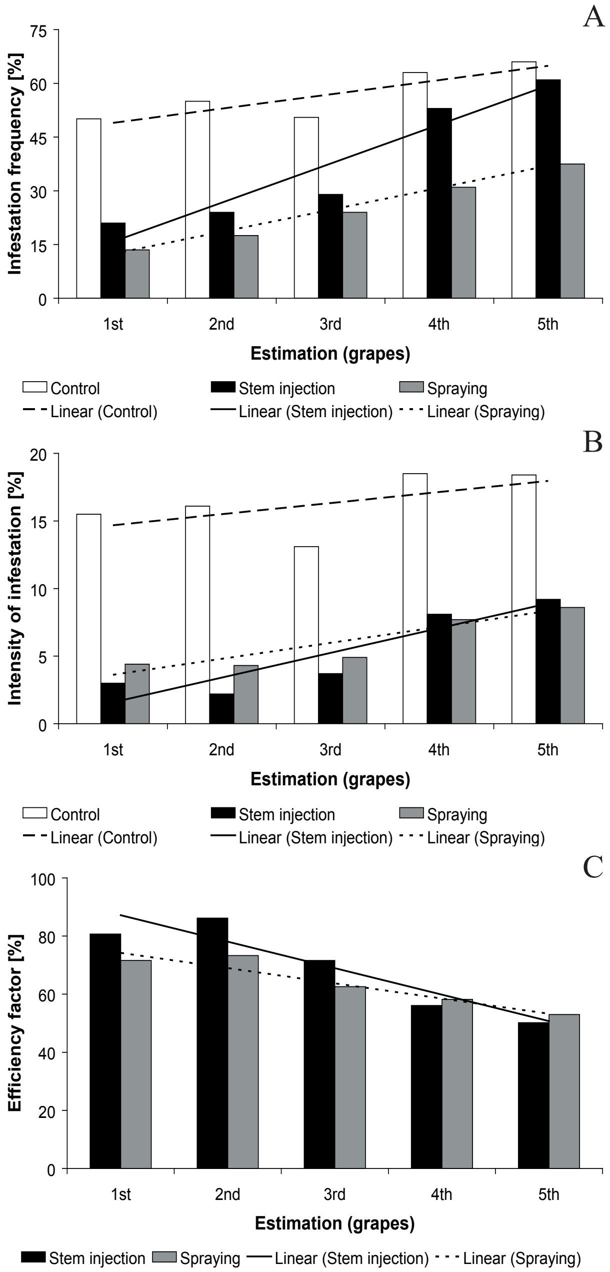

Fig. 3: Results of the repeated xylem injection of non-formulated metalaxyl with a prototypal long-term injection system compared with the simultaneous spraying of the formulated product (Fonganil Gold®). The values were determined from the estimations of 200 (control plants, sprayed application) and 100 grapes (stem injection) per variant. The regression lines were generated using Microsoft Office Excel 2003. **A**) Infestation frequency of downy mildew on the grapes of test variants and control plants. **B**) Intensity of infestation with downy mildew on the grapes of test variants and control plants. **C**) Efficiency factors of the test variants.

ronment by means of drift, volatilisation, wash off, infiltration and driftage. Stem application is therefore an ecologically sound method of plant protection that would primarily be suitable to protect plants in the vicinity of bodies of water or on the outskirts of towns.

Due to the method's current stage of development, it is not yet possible to carry out a realistic calculation of costs involved and to compare them with spraying methods conventionally applied in viticulture. The disadvantage of the method presented here is that high acquisition costs are involved, even if this would decrease considerably in the event of serial production. The material costs of the inlet and needle system to build the prototype, for instance, totalled approx. 9 Euros per grapevine. The costs were high because the needle holders were custom-made. Injection-moulded serial production would reduce costs to around 40 cents per grapevine. The economic advantage of stem application arises from the lower expenditure of human labour involved n applying the plant protection agent. Due to the central loading of the system the rows of grapevines do not need to be passed through during the spraying process, saving a lot of time.

R e s u m e : These tests have demonstrated that it is fundamentally possible to carry out grapevine protection by means of stem injection. Efficiency factors of practical relevance (> 70 %) were achieved by injecting metalaxyl. The protective effect could be proven for both leaves and grapes. Extensive anti-resistance management, however, first requires the location of other agents suitable for use in stem injection.

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