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ORIGINAL PAPER

Impact analysis of different applications of cyantraniliprole on control of horse chestnut leaf miner (*Cameraria ohridella*) larvae supported by biophoton emission

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Abstract
Cameraria ohridella is one of the most invasive pests of horse chestnut. Cyantraniliprole is one of the most perspectively active insecticides, which can transport within the plant in several ways, and its efficacy against this pest has not yet been tested. All three modes of application were effective against the target pest, but there was a difference in the time of action between them. However, no demonstrable difference in the speed of action was detected between the doses used. A more intense rate of acropetal translocation was confirmed compared to basipetal translocation. A trend-like effect between the applied concentration of cyantraniliprole and the photon emission intensity per unit area of plant tissue was observed in the translaminar and acropetal treatment settings. In both cases, a clear increase in photon emission was observed, indicating metabolic upregulation. Therefore, we can conclude that biophoton emission measurements can be utilized to conduct efficient pesticide translocation investigations.

Keywords Cyazypyr · Horse chestnut leaf miner · Chemical control · Application method · Biophoton emission

Introduction

The horse chestnut leaf miner, *Cameraria ohridella* Deschka & Dimić, 1986 (Lepidoptera: Gracillariidae), originates from remote natural stands of the European horse chestnut, *Aesculus hippocastanum* from Balkan peninsula (Grabenweger and Grill 2000). It was first observed attacking ornamental horse chestnut trees in Macedonia in the 1970s, from where it spread to most part of Europe. Since then, in all invaded regions, outbreaks have continued unabated, causing aesthetic damage to horse chestnut, one of the favorite ornamental trees in European cities (DAISIE 2022).

The main host plant of *C. ohridella* is the horse chestnut, in the leaves of which it lives. The consequence of its damage is the spreading of mine on a leaf plate. Mine formation is accompanied by the gradual loss of photosynthetic tissue, clearly leading to completely dead necrotic leaf parts, similar to the frequently occurring hypersensitive responses of plant tissues (Balint-Kurti 2019). This latter may constitute a substantial part of the photosynthetic tissue, thus leading to severely damaged plant metabolism at a later stage of the damage, that leads to necrosis and the total drying of the leaf tissue, because of which the injured leaf eventually falls from the tree (Salleo et al. 2003). Due to its defoliating damage, it is the most important arthropod pest of this ornamental tree. Besides, successful development of this pest is also occasionally observed in *Acer pseudoplatanus* and *A. platanoides*. It also develops in some species of the genus *Aesculus* (Skuhravy 1998; Freise and Heitland 1999).

C. ohridella develops two-four generations per year and overwinters in the pupal stage in defoliated, dead leaves. The emergence of its adults in spring occurs between the beginning of April and the second half of May, depending on climatic conditions (Gilbert et al. 2003; Girardoz et al. 2007). After mating, each female lays about 180 eggs on the upper part of the leaves (Girardoz et al. 2007). Larval development lasts 25–35 days (Skuhravy 1998; Freise and Heitland 1999). First-instar larvae make only a small gallery. The second- and third-instar larvae develop a round mine of 4–7 mm in average diameter.

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Among several chemical application methods, the trunk injection of *A. hippocastaneum* is widely used to control *C. ohridella*. Good efficacy has been ensured with systemic injection (endotherapy) of abamectin and imidacloprid in practice (Ferracini and Alma 2008), although the use of these systemic insecticides is the subject of some concern. This invasive method of applying the pesticides via the trunk could damage the tree by creating a potential entry for xylem pests, mainly fungal disease agents (Schweingruber 1988). Moreover, these insecticides distributed via vascular tissues to leaves could directly control pests, but its presence in other plant organs, such as flowers, could harmfully touch the population of pollinators (Whitehorn et al. 2012).

Cyantraniliprole is a novel anthranilic diamide insecticide that will enable to control of several key herbivore pests (Lahm et al. 2009; Jeanguenat 2013). A large number of field, greenhouse and laboratory studies in many countries have shown that these active ingredient-containing products are very effective against several species of both chewing and sucking pests in plant cultivation (Mandal 2012; Misra and Mukherjee 2012; Rath and Nayak 2013). Therefore, it can be successfully used against caterpillars, thrips, whiteflies and some aphid species.

Cyantraniliprole is part of Group 28 according to the IRAC mode of action classification scheme (Jeschke 2017). Anthranilic diamides have a special mode of action during which selectively activates insect ryanodine receptors causing mortality from uncontrolled release of calcium ion stores in muscle cells (Shelby et al. 2013). The additional unique character of this active ingredient is many kinds of translocation way in plant tissues. So, it can go in both acropetal, basipetal and translaminar ways to the target pest (Barry et al. 2015). Intoxication symptoms include rapid feeding cessation, general lethargy, paralysis, regurgitation (lepidopteran larvae) and eventual pest mortality (Dong et al. 2017).

The unique properties of cyantraniliprole allow selective control of pests while conserving beneficial arthropods. According to its novel mode of action reinforced by laboratory experiments, it has shown equal performance against susceptible and resistant pest populations (Lahm et al. 2009; Costa et al. 2020). Those attributes can make cyantraniliprole a valuable element of integrated pest management (IPM).

The life activity of hidden-lifestyle arthropod pests and the stress phenomenon caused by their damage to the plant is spontaneously bioluminescent and can be detected by the Night Shade LB 985, similarly to previous works (Lukács et al. 2022; Pónya et al. 2022), when the damage caused by some biotic stressors, i.e., *Oulema melanopus* and *Tetranychus urticae,* was possible to detect and follow by this non-invasive technique. Since the intensity of spontaneous plant bioluminescence is extremely low, its detection requires special and sensitive instrumentation. Usually, light-scattering tubes and photon counting devices are used for this purpose (Kobayashi et al. 2014). Based on all this information above, the main objective of this research was to obtain data in connection with the mortality effects of cyantraniliprole applied in different ways against *C. ohridella*.

Additionally, a further goal was to characterize and visualize the change of plant stress phenomena after the insecticide treatment by means of biophoton emission. Thus, we hope to be the first to obtain data regarding the effects of this active ingredient against this important leaf-mining species by examining the output of this modern imaging method, as well as the changes in the photon emission-related metabolic processes of the damaged leaf of the host triggered by cyantraniliprole.

Materials and methods

Sampling and experimental setting

To determine the insecticide efficacy of different cyantraniliprole applications, horse chestnut leaves damaged by *C. ohridella* were collected from insecticide-free environment. The collection was carried out in early September 2022 in the Karád area (Hungary, Somogy county, GPS coordinates: 46°69′07.60" N 17°84′13.60" E). We have collected such damaged leaves in which we found living larvae, which criterion was judged by the precise movement of larvae found in leaves facing the light.

The collected leaves were placed in Petri dishes between blotting paper soaked in water, and the samples were immediately subjected to experimental treatment. Cyantraniliprole [100 OD] (oil dispersion)] was tested in three different concentrations: 5, 10 and 30 ml a.i. ha^{-1}. Each concentration was applied in translaminar (dropped directly on the larvae mining into the leaves), basipetal (dropped onto the leaf surface 2, 4 and 6 cm from the mining larvae) and acropetal ways (the petioles of the leaves are soaked in the prepared solutions for 3 h) (Fig. 1). Beside the untreated controls, each evaluated dose of each application way was set up with six replications each.

Subsequently, triggered mortality was evaluated at every hour for 10 h, and thereafter every 3 h for 2 days after the insecticide treatment. Between the experimental evaluations, the samples were put into an incubator. The conditions set in the incubator [temperature: 25 °C and (15L:9D) photoperiodic parameter] provided the typical conditions in Hungary at that time of the year.

In parallel, we measured the biophoton emission of treated and untreated samples under laboratory conditions during the mentioned exposure times in order to determination of the plant stress change.

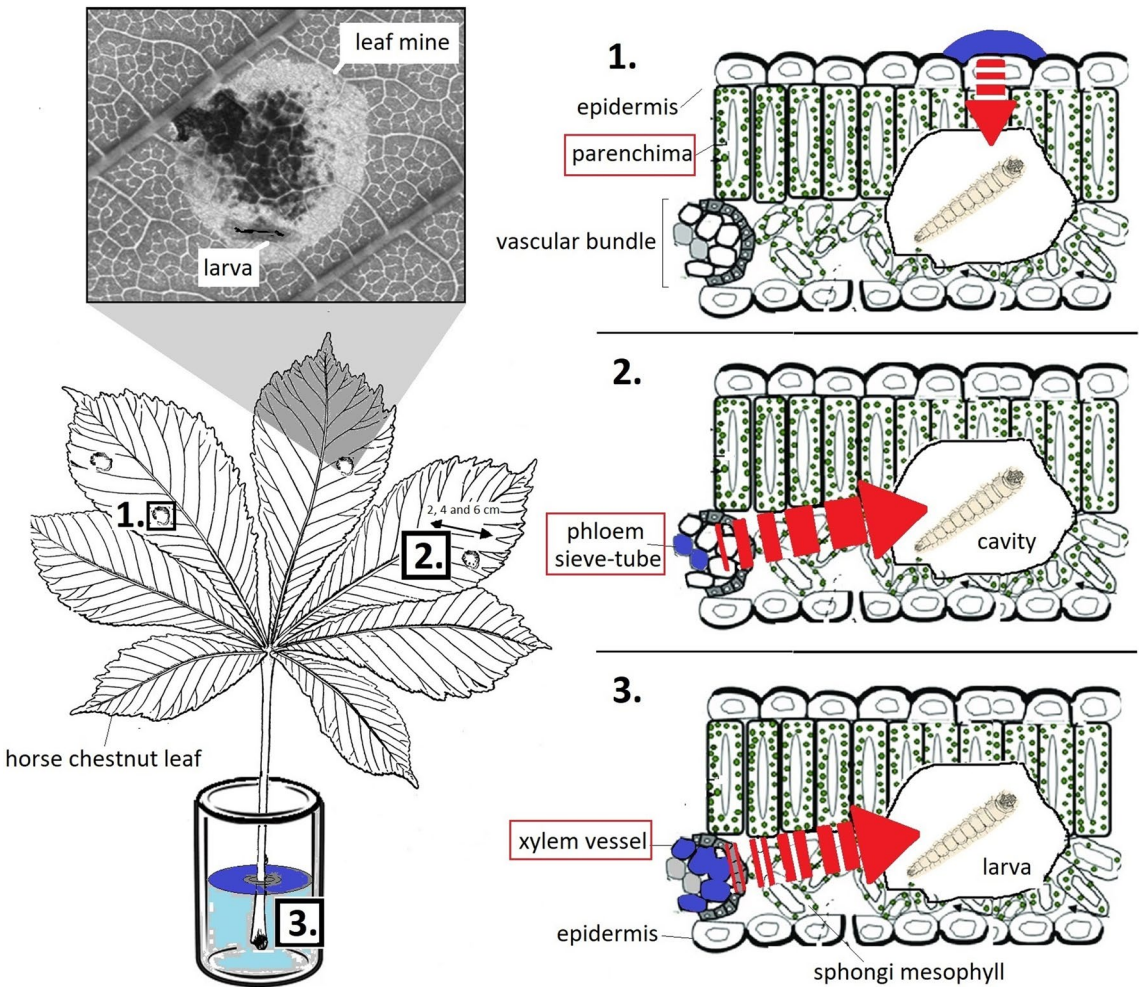

Fig. 1 Scheme of different application ways of cyantraniliprole against *C. ohridella* based on the work of Ustin and Jacquemoud (2020) 1. translaminar, 2. basipetal, 3. acropetal, blue: suspension of the insecticide, red frame: place of the insecticide translocation; red arrows: direction of translocation

Assessment of registered mortality

At the end of each exposure, the mortality of the larvae and pupae was determined by placing them on the lighted glass of the treated leaves. The judgement of mortality was based on the motility of the semaphoronts, which was also confirmed by tissue dissection. We have determined the average time (SE) of mortality triggered by different treatments using the noted data of each lethality period (min).

Moreover, the average distance (s) of the treatments from the center of the leaf mine (in the case of acropetal treatments, on average 200 mm, while in the case of basipetal treatments 20 mm) and registered mortality periods (t) (min) allowed the determination of the translocation speed ($v = s/t$; in mm/min) of the insecticide.

Biophoton measurement-based stress assessment assay

The NightShade LB 985 in vivo plant imaging system (Institute Berthold Technologies Bioanalytical Instruments, Calmbacher Strasse 22, D-75323 Bad Wildbad, Germany) was used for the measurement and detection of bio-photon emission. The instrument developed for bioluminescence measurements is equipped with a sensitive, slow-scanning, thermoelectric Peltier-cooled NighOwlcam CCD (Charge Coupled Device). This instrument is controlled by IndiGo™ 2.0.5.0 software. The images captured by the camera and saved by the software allow the isolation of pixel intensity peaks, which will determine the luminescence during the evaluation process. Since the setup parameters were constant

throughout the whole period of the measurement, the variations in the relative pixel intensities obtained reflected the photon emission generated by the treatment, the value of which was converted into "counts per second (cps)" by the analysis software. The integration time was set to 60 s with a 4 × 4 binning factor. Simultaneous "background correction" and cosmic ray attenuation were performed during image acquisition to eliminate high intensity gamma rays. Immediately after placement in the dark chamber, leaf bioluminescence measurements were initiated, and the delayed fluorescence (DF) signals were recorded for ten minutes.

Biophoton emission recordings were conducted on damaged horse chestnut leaves after translaminar, basipetal and acropetal treatment with cyanantraniliprole at concentrations of 5, 10 and 30 ml a.i. ha^{-1}. These non-invasive measurements were performed uniformly at 1.5, 3, 7, 18, 54 and 48 h after pesticide application.

During the evaluation of the results, the nectrotic parts of the mines created by the insect damage were taken into consideration, as it was crutial to objectively assess the biophoton emission signals emitted from the still functional part of the leaf. For this reason, a correction was applied to the intact plant tissue (Fig. 2). During this operation, pixel points of the dead areas were selected using GIMP 2.10.8 software and the true photosynthetic surface area was determined based on the knowledge of the damaged leaf surfaces. The calculated value was then used, and measurements were made to determine significance.

Statistical analysis

In order to test the mortality data of leaf miner larvae ($n^{>}50$), the Shapiro–Wilk test was used. For the survey of the normal distribution of data ($p < 0.05$), the Ghasemi- and Zahediasl-type methods were employed. One-way ANOVA and principal coordinate analysis (PCoA) based on Euclidean distance were performed to compare the effect of times elapsed since treatment and dose rate of cyantraniliprole on larvae mortality. Means were separated by using the Tukey (HSD) test, at $p \leq 0.05$. Statistical calculations were carried out using SPSS for windows (version 20.0; SPSS Inc. Chicago, IL) and NuCosa software package (Tóthmérész 1993).

Results

Analysis of the duration of different treatments inducing insect mortality

Our study has demonstrated that there is a fundamental difference in the insecticidal activity of cyantraniliprole against different developmental stages of *C. ohridella*. The doses of the active substance analyzed in all treatments included in the trials resulted in 100% mortality on developing larvae. Our observations confirmed that the larvae stopped feeding and their previously intense movements were reduced after the treatments, until complete sessility

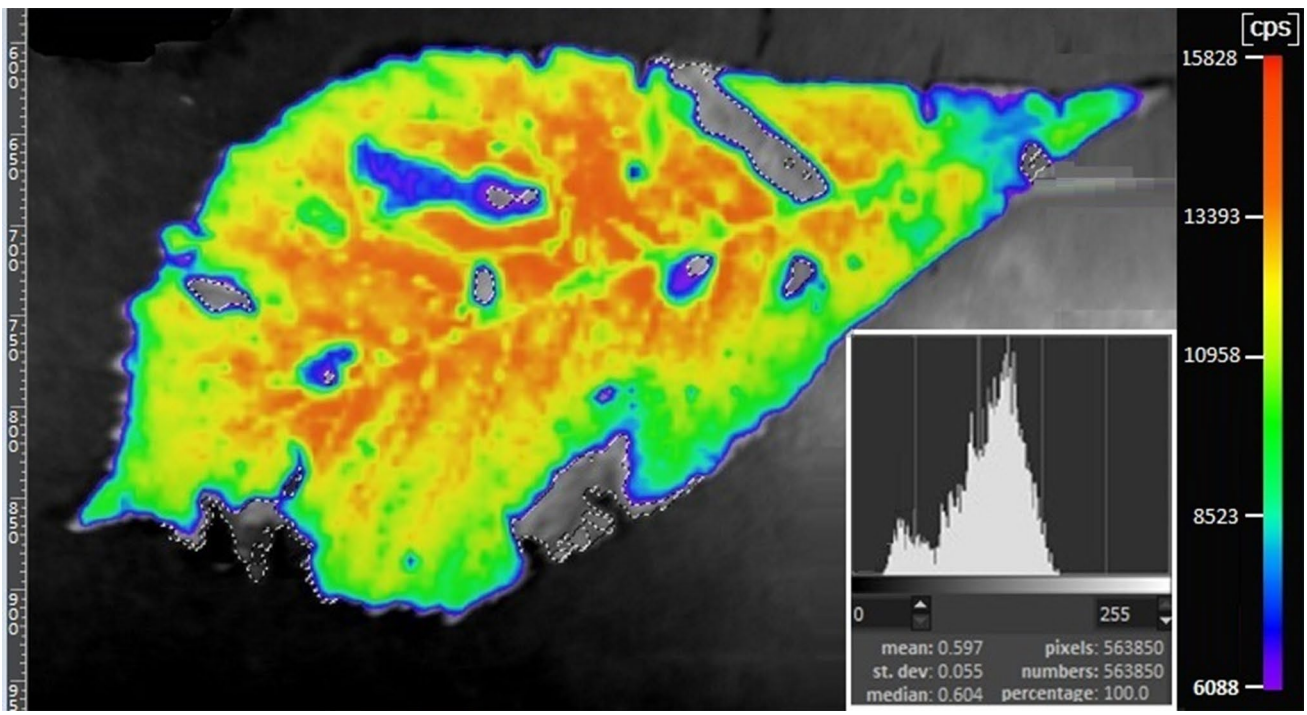

Fig. 2 Leaf surface destruction caused by *C. ohridella* with the help of GIMP software

and then death. In the pupal stage, no such difference in movement intensity was detected. No mortality of pupae was recorded during post-treatment monitoring.

Figure 3 shows the duration of mortality induced in larvae by different applications of cyantraniliprole. The one-way ANOVA revealed that there was a statistically significant difference in insecticidal effect of different doses of examined applications [5 ml a.i. ha^{-1}: $F\ (1.22) = 178.06$, $p = 5.04 \times 10^{-12}$; 10 ml a.i. ha^{-1}: $F\ (1.22) = 15.96$, $p = 0.0006$; 30 ml a.i. ha^{-1}: $F\ (1.22) = 19.91$, $p = 0.0001$]. The principal coordinate analysis (Fig. 4.) showed the grouping of different applications as a function of times elapsed since treatment. These results show that translaminar, basipetal and acropetal treatment is statistically distinguishable from each other. When considered together, the acropetal treatments killed larvae developing in the leaf miner in an average of 2416.66 min. In contrast, the translaminar treatments induced target organism lethality in 316.66 min, while the basipetal applications in 293.33 min. There was no statistically significant difference between the mortality-inducing duration of the latter two applications ($p = 0.145$).

Among different doses of acropetal applications, the 10 ml a.i. ha^{-1} treatments resulted the slowest effect on the insect. Treatments at 5 and 30 ml a.i. ha^{-1} killed those larvae that were developing in the leaf miner in almost the same time. However, no statistically verifiable difference could be detected in the mean mortality-inducing durations of these two doses ($p = 0.287$).

The mortality durations of different doses of basipetal insecticide applications were not statistically proved to differ ($p = 0.162$). Different doses of cyantraniliprole treatments applied at different distances from the insect mine killed larvae in nearly the same time. Statistically significant differences were recorded for all three doses at insecticide depending on insect-mine distances applications [$F\ (1.16) = 65.26$, $p = 4{,}89 \times 10^{-7}$]. The time to kill the target organism was approximately twice as long for the adjusted leaf treatments at 6 cm from the leaf mine compared to treatments at 2 and 4 cm.

The lethality-inducing duration of translaminar treatments was shorter for the higher doses applied. Thus, the 30 ml a.i. ha^{-1} treatment produced the fastest effect. However, the difference in mortality-inducing durations

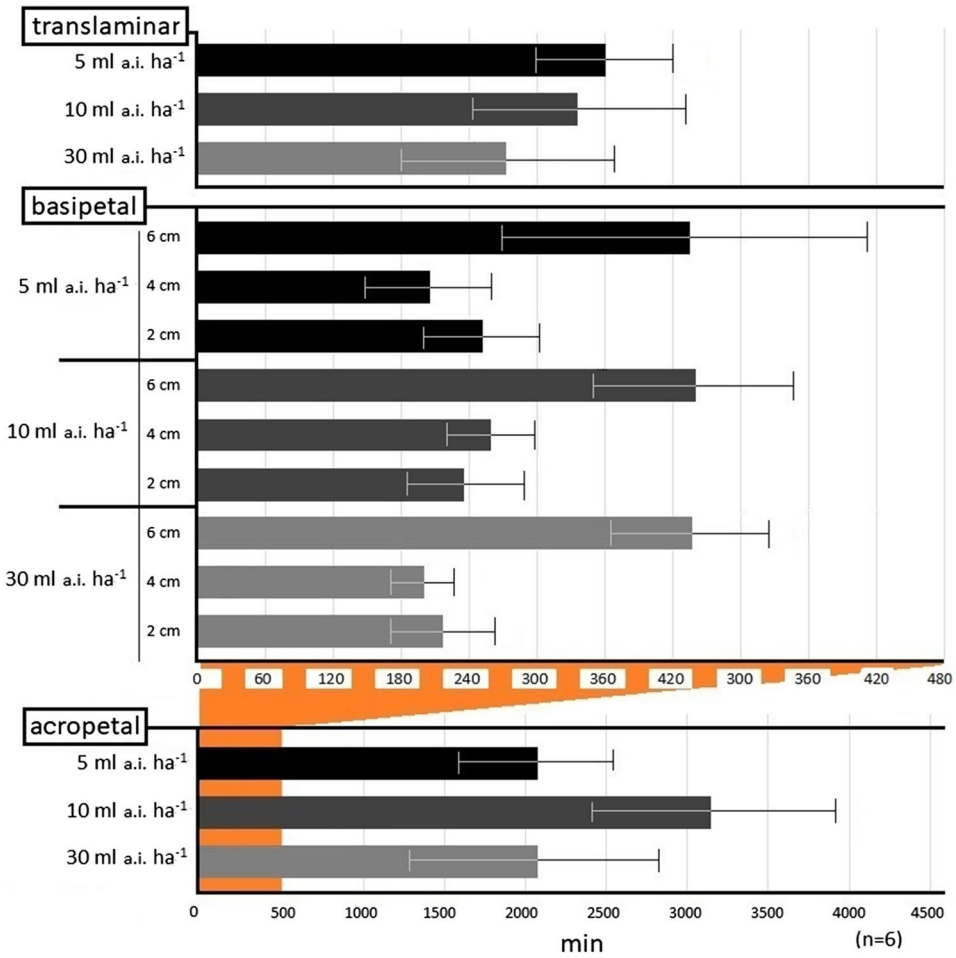

Fig. 3 Mean application durations (min ± SE) for different cyantraniliprole applications and doses inducing mortality of *C. ohridella* larvae ($n = 6$ for each group)

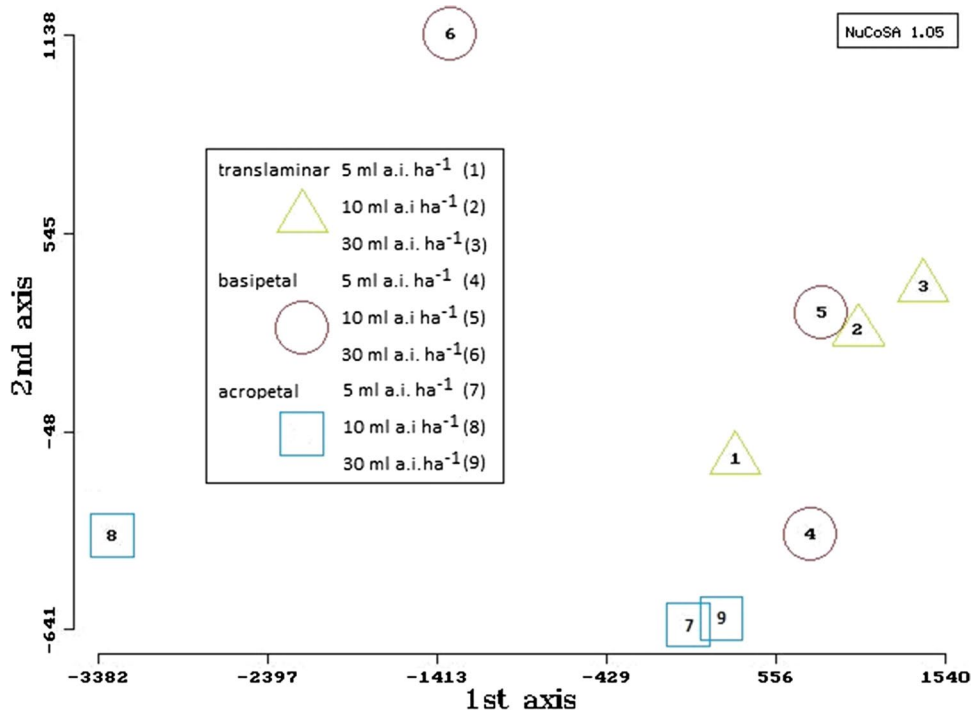

Fig. 4 Principal coordinate analysis (PCoA) based on Euclidean distance showing the distribution of effect of times elapsed since treatment and dose rate of cyantraniliprole on larvae mortality (Axis 1 = 0.8625; Axis 2 = 0.9876)

Table 1 Average insect mortality rates of basipetal (insecticide applied at 2 cm from the mine) and acropetal applications of cyantraniliprole and different doses (mm/min ± SE)

	basipetal	acropetal
5 ml a.i. ha^{-1}	0.811 ± 0.162 mm/min	1.481 ± 0.012 mm/mi
10 ml a.i. ha^{-1}	0.851 ± 0.057 mm/min	0.997 ± 0.136 mm/min
30 ml a.i. ha^{-1}	0.930 ± 0.084 mm/min	1.481 ± 0.094 mm/min

of translaminar treatments at different doses was not statistically verified ($p = 0.117$).

When the duration of insect mortality induced by different doses of different treatments is examined as a function of insecticide translocation distances, the opposite results to those described above are obtained (Table 1). It can be seen that acropetal (translocation from the base to the apical direction through the xylem elements) transport was faster than basipetal (translocation from the apical to the basal direction through the phloem elements) transport for all three doses used. These measured differences were confirmed by the results of statistical tests, both in the case of doses [F (1.16) = 15.38, $p = 0.001$] and application methods [F (1.34) = 8.31, $p = 0.006$]. The translocation of the cyantraniliprole drug through xylem elements is about 1.52 times faster than the translocation through the phloem bundles.

Analysis of the duration of different treatments on the emitted delayed fluorescence signals

Figure 5 shows the biophoton emission (cps) values of the tested leaves subjected to different cyantraniliprole treatments. The examination confirmed the effects of the active substance cyantraniliprole in plant metabolic reactions. The higher biophoton emission values of the insecticide treated samples compared to control samples are a good representation of the effect of the tested pesticide on plant metabolism.

At 1.5 and 3 h from the translaminar treatment setup, the biophoton emission measured at 5 and 10 ml a.i. ha^{-1} concentrations both exceeded the control value and resulted in an increase. For the 30 ml a.i. ha^{-1} cyantraniliprole, the 3, 7 and 18 h treatments exceeded the photon emission intensity of the control. After 7 h of treatment, the biophoton values exhibited only little variation irrespective of the dose. In addition, we observed a continuous decrease in the level of biophoton emission with increasing cyantraniliprole concentrations and recording time. The average photosynthetic surface area reduction was 24.01 ± 3.08%. The one-way ANOVA revealed that there was a statistically significant difference in variation in the biophoton emission values as a function of exposure times for all three applied translaminar doses [F (1.46) = 65.30, $p = 2.25 \times 10^{-10}$].

In the basipetal experimental setting, a plant stress response was detectable at 3, 7 and 18 h for 5 ml a.i. ha^{-1}. Leaves treated with 30 ml a.i. ha^{-1} cyantraniliprole showed a measurable increase at 7 and 18 h. For 10 ml a.i. ha^{-1}, a measurable difference was observed at 18 h after the

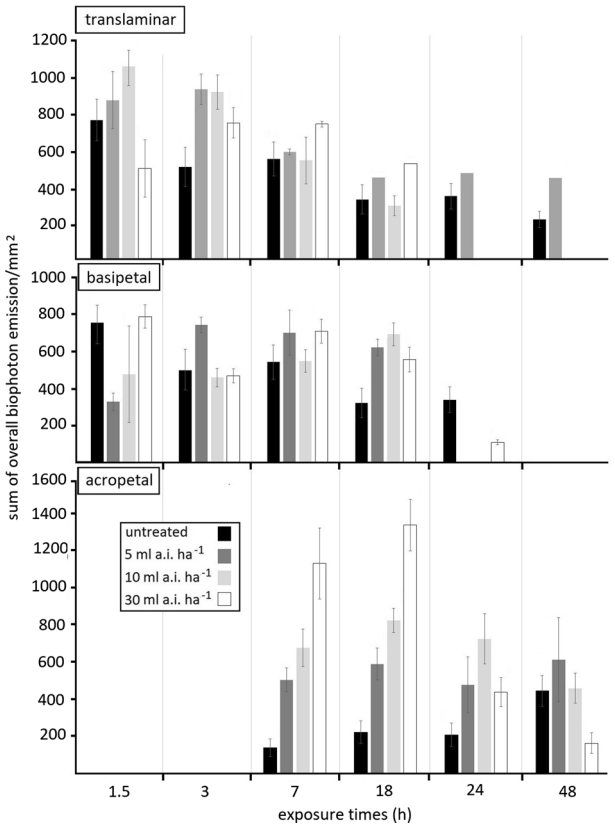

Fig. 5 Results of biophoton emission analysis (mean cps ± SE) after different cyantraniliprole applications and doses ($n = 6$ for each group)

experiment was set up compared to leaves treated as control. Measurements prior to this did not show any trend differences. Experimental leaves treated with 5 ml a.i. ha^{-1} and 30 ml a.i. ha^{-1} of the active substance concentration in the basipetal manner showed statistically significant differences in biophoton emission values as a function of exposure times. In contrast, for leaves treated with 10 ml a.i. ha^{-1} cyantraniliprole, this correlation was not statistically confirmed ($p = 0.231$).

The most pronounced effect of the active substance cyantraniliprole could be detected for the acropetal treatments. In this case, significant differences in biophoton emission values were observed even after 24 h. As in the translaminar study, a decrease was observed in the acropetal experimental setting with increasing treatment duration from 24 h onwards. In the acropetal setting, there is no statistically significant difference between the measured biophoton emission values and the experimental time periods when using a concentration of 5 ml a.i. ha-1 active ingredient ($p = 0.075$). However, when using higher concentrations of cyantraniliprole, the one-way ANOVA revealed that there was a statistically significant difference in the chemiluminescence due to the plant stress response and the exposure times tested [10 ml a.i. ha^{-1}: $F(1.30) = 301.78$, $p = 3.33 \times 10^{-17}$; 30 ml a.i. ha^{-1}: $F(1.30) = 39.77$, $p = 5.93 \times 10^{-7}$].

Figure 6 shows the decay of fluorescence in horse chestnut leaves. The minute-to-minute variation in fluorescence is clearly observed from the recordings.

Discussion

Our study clearly demonstrated the rare intra-plant multidirectional translocation of cyantraniliprole. In all leaves evaluated, the death of treated insect larvae was detectable, demonstrating the absorption and translocation of the active substance. The long-lasting residual effect of cyantraniliprole has already been reported by Pes et al. (2020) in their study on *Delia platura* (Meigen) and *Ostrinia nubilalis*

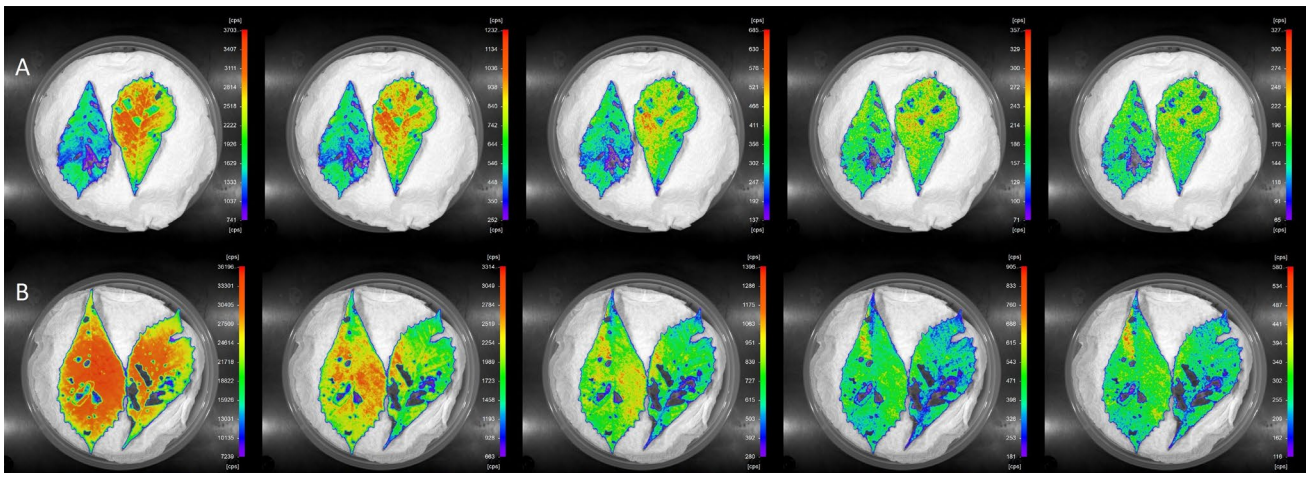

Fig. 6 Decreasing character of delayed fluorescence of untreated and cyantraniliprole treated leaves as a function of time. A: untreated, B: treated by cyantraniliprole in translaminar way

(Hübner). The acro-, basipetal and translaminar transport of this systemic compound and its toxicity to target pests have also been reported in several previous studies (Cloyd et al. 2011; Sidhu et al. 2014; Chen et al. 2015; Schmidt-Jeffris and Nault 2016).

The selectivity and target site activity of this insecticidal active substance is demonstrated by the radically different insecticidal activity recorded in relation to different emergence stages of *C. ohridella*, as larvae and pupae. Regardless of the treatments applied, the absolute efficacy (100%) recorded for treated larvae and the lack of insecticidal activity observed for treated pupae were observed. The toxicity of cyantraniliprole on eggs and larvae (Hardke et al. 2011; Tiwari and Stelinski 2013) and reduced (Wang et al. 2019), sometimes absent pupae (Mandal 2012; Dong et al. 2017) has been confirmed in several studies on several insect species. The narrow spectrum of activity of the compound is also evidenced by its recorded selectivity for beneficial organisms, as described by Wang et al. (2019) using the example of *Encarsia formosa*, a natural enemy of greenhouse molting insects, among others. Its environmental friendly feature is complemented by its lack of acute toxicity to warm-blooded animals (Lahm et al. 2009), which, among other things, reflects the target site activity of this diamide molecule.

Our study confirmed the translaminar translocation of cyantraniliprole in horse chestnut leaves, a movement within plant tissue that was also confirmed by the results of parallel studies (Tiwari and Stelinski 2013; Barry et al. 2015). This translocation ability was demonstrated in the presence and absence of adjuvants applied, based mainly on the results of acute toxicity studies in *Hemiptera* species.

Our observations confirmed a more intense rate of acropetal translocation relative to basipetal translocation. This observation is in agreement with the results of Barry et al. (2015) and Pes et al. (2020), who found that compared to the significant acropetal translocation of the analyzed diamide molecule, its basipetal translocation is much lower. They pointed out that in seed treatments, insecticidal translocation increased larval mortality even at lower concentrations compared to foliar spraying. Moreover, the prominent acropetal nature of this active ingredient through xylem elements is confirmed by several seed treatment experiments (Thrash et al. 2013; Wilson et al. 2021).

In terms of biophoton emission studies, the present experimental setup is considered novel. To date, no studies have been conducted on the combined effects of mine forming into leaf triggered by a hidden-lifestyle pest and the active ingredient cyantraniliprole on changes in delayed fluorescence values. Based on our results, we observed a trend-like effect between applied concentrations of cyantraniliprole and photon emission intensity per unit area (mm^2) of plant tissue in the translaminar and acropetal treatment settings. In both cases, a clear increase in photon emission was observed in the first two measurement times, proportional to the applied concentration, which trend disappears in the last two measurement times of the experiment, and a significant decrease in signal intensity was also observed.

The non-invasive measurement of bioluminescence has been previously shown to be suitable for the assessment of biotic stress (Florvszak-Wieczorek et al. 2011; Lukács et al. 2022; Pónya et al. 2022). In our studies, photosynthetic processes gradually started to shut down after the plants were placed in the dark chamber, resulting in electron backflow and chlorophyll excitation in photosystem II and inducing delayed fluorescence. The decay of this phenomenon reflects, in practical terms, the health or the stress level of the plants (Jia et al. 2020), with higher initial values indicating a healthier plant state, which is supported by literature data. Gerhardt and Bodemer (2000) described that 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) treatment can be used to distinguish between higher emissions of a healthy plant and DF emissions of a treated plant. In addition, a methodologically important aspect of our results is that biophoton emission measurements can be used to perform effective translocation studies. However, the decrease in signal intensity over time suggests that detached leaves can only be accurately examined for a limited time period, in our experiments 18 h for translaminar and 24 h for acropetal treatments, which should be an important element of the experimental methodology for all plant species under investigation.

Taking into account our results, it can be said that the acropetal experimental setting has the greatest effect on plant metabolism when the active ingredient cyantraniliprole is applied. The reason for this may be that the acropetal experimental setting modeled natural plant translocation processes the best among the three ways of translocation studied here.

Huynh et al. (2021) studied in detail the persistence and metabolism of cyanantraniliprole in tomato plants and found that the active substance is both translocated to different parts of the plant and metabolized at decreasing concentrations and below the threshold, but different metabolized forms are also found in ripening tomato fruit. However, Vuković et al. (2021) studied the effect of cyantraniliprole on peach leaves after 7 days of treatment and found decrease in chlorophyll concentration, along with increased carotenoid level. To the best of our knowledge, no studies have been carried out to date to investigate its short term, hourly, effects on leaves. Therefore, our results are of importance from the perspective of the physiology of the plant in addition to elucidating the efficacy of the agent against the pest and the effectiveness of translocation pathways. The photon intensities emitted in the first phase of the experiment and their increasing tendency along with

the applied concentration suggest that cyantraniliprole has an effect on plant metabolism, probably on the functioning of photosynthesis and/or on the formation of reactive oxygen species (Jócsák et al. 2020; Lukács et al. 2022; Pónya et al. 2022). Our data indicate that this insecticide may have short term stimulatory effect on plant responses, which can be inferred from the increasing delayed fluorescence value. It is a common phenomenon of the initial part of stress, or low level of stress that is called 'eustress', in the case of which there is an overall metabolic upregulation that manifests, e.g., in the increase in pigment content (Abdel et al. 2019). The results of related work on the decay of DF indicated that stress conditions initiate sharp decrease in delayed fluorescence. According to the work of Razinger et al. (2012), DF significantly decreased in 140 µM DCMU and 100 µM methyl-viologen (MV), i.e., paraquat-intoxicated potato leaves during 5 and 72 h experiments: both 100 µM $CuSO_4$ and 100 µM $CdCl_2$ induced significantly steeper decline of DF than that of the control (Razinger et al. 2012). However, further analytical studies (free radical staining, antioxidant assay, lipid oxidation assay) are needed to elucidate this phenomenon.

Our study also demonstrated that the active substance cyantraniliprole, a member of the diamide group, is an unique active substance with target site activity that provides protection against the damaging form of the target organism. In addition to this target site activity, it does not affect other non-pestiferous stage of the pest. Although the binding of ryanodine to the receptor site may make it toxic against other motile forms, its unique selectivity makes it an excellent tool for Integrated Pest Management (IPM) to ensure sustainable development. A further advantage, supported by literature data, is the prevention of resistance or even cross-resistance (Bielza and Guillén 2015) in target organisms by widely used neurotoxins, which is explained by its entirely new mechanism of action.

Overall, our experimental results have provided substantial support for the activation of cyantraniliprole against *C. ohridella*, with the potential for extending the registration of the compound to a wider range of applications as a control of ornamental and public trees against acaricidal and other pests. The realization of these future objectives, however, will require further impact studies. The use of the compound against *C. ohridella* is most likely to arise by trunk injection, which is explained by its proven excellent acropetal transport. However, such an application may raise concerns about pollinator exposure, which should be clarified. Among other things, the toxicity of the compound to other organisms, including beneficial organisms and pollinators, and the extent of its impact on the environment when used under these conditions are not known. Without the exclusion of these important undesirable side effects, the widespread practical use of this promising insecticidal active substance is not feasible. This study may shed some light on the broadening of the field of application of this compound, which may even in the future be an effective tool for the successful control of many hidden lifestyle threat insect pests.

Conclusions for future biology

Few effective plant protection products are available today to successfully control *C. ohridella*. The cyantraniliprole insecticide we are testing is undoubtedly an effective and promising option for future pest management. Based on our results, we believe that acropetal transmission, i.e., application by trunk injection, can be the most dynamic and environmentally friendly method. The basipetal and translaminar routes, i.e., spraying on the leaf surface, are also effective. Future use of the compound in this way, however, should be preceded by a number of further environmental studies, including the impact on honey bees and other beneficial organisms.

A further novel observation of our study is the demonstrated plant life processes effect of cyantraniliprole. The more intense photosynthetic activity of the treated leaves certainly indicates this. This phenomenon is already known for some fungicidal active substances. However, a similar plant life-cycle side effect of insecticides is less well understood. The other effects of insecticides on plant organisms raise a number of questions, for the discovery of these positive and possible negative consequences requires further additional experiments. Overall, a more thorough exploration of this area may offer many new developments in plant physiology and protection disciplines in the future.

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Declarations

Conflict of interest The authors declare that they have no potential conflict of interest in relation to the study in this paper.

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