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Predatory mites (Acari: Phytoseiidae) on wild blackberry in Norway

Results from a search for *Amblyseius andersoni* in August 2016

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Nina Trandem, Karin Westrum, Anette Sundbye, João Pedro I. Martin, Gilberto J. de Moraes
Divisjon for bioteknologi og plantehelse

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FORFATTER(E)/AUTHOR(S)

Nina Trandem, Karin Westrum, Anette Sundbye, João Pedro I. Martin, Gilberto J. de Moraes

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SAMMENDRAG/SUMMARY:

Rovmidden *Amblyseius andersoni* er ønsket som ny nytteorganisme mot skadedyr i norske hagebruksvekster. Arten ble aldri funnet av Torgeir Edland, som undersøkte norsk rovmiddfauna på åtti- og nittitallet. Ettersom den er funnet på bjørnebær i Sverige og Danmark, og et mildere klima kan ha endret forholdene for arten siden Edlands studier, gjorde vi i 2016 et rettet søk etter *A. andersoni* i ville bjørnebær (*Rubus tomentosus*, sensu lato). Nesten 1500 potensielle rovmidd (Acari: Phytoseiidae) ble funnet på ca. 550 bjørnebærblader samlet ved Sandefjord, Grimstad, Fredrikstad og Ås. Over en tredjedel av middene ble undersøkt ved Laboratory of Acarology ved Universitetet i São Paulo (Brasil). A. *andersoni* ble ikke funnet, men derimot minst 10 andre arter Phytoseiidae, alle kjente fra Norge tidligere. Resultatene våre støtter tidligere undersøkelser som tyder på at *A. andersoni* ikke finnes naturlig i Norge. For en sikrere konklusjon gir vi noen råd om videre leting.

The predatory mite *Amblyseius andersoni* (Acari: Phytoseiidae) is wanted as a new biocontrol product in Norwegian horticulture. The species was never found by Torgeir Edland, who surveyed the Norwegian fauna of phytoseiids for more than 20 years. Since *A. andersoni* has been found on blackberry in both Sweden and Denmark, we did a specific search for it in wild blackberry (*Rubus tomentosus*, sensu lato) in 2016. Almost 1500 potential phytoseiids were found on about 550 blackberry leaves collected near Sandefjord, Grimstad, Fredrikstad, and Ås. More than a third of these were examined at the Laboratory of Acarology (University of São Paulo, Brazil). *Amblyseius andersoni* was not found, but at least 10 other species of Phytoseiidae, all previously reported from Norway, were present. Thus, our survey supports earlier ones, indicating that *A. andersoni* is not naturally occurring in Norway. We conclude with some suggestions for an extended search.

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INGEBORG KLINGEN, HEAD OF DEPARTMENT **INVERTEBRATE PESTS AND WEEDS IN FORESTRY, AGRICULTURE AND HORTICULTURE**	**ANETTE SUNDBYE**

Preface

This report summarizes the results from a specific search for the predatory mite *Amblyseius andersoni* made in 2016. The work was commissioned by a project on increasing the use of biological control products in Norway, with Anette Sundbye as the project leader. The project was funded by the Norwegian Agriculture Agency and the Norwegian Ministry of Agriculture and Food, through the National action plan for sustainable use of plant protection products.

The leaf sampling was carried out by Karin Westrum, Geir K. Knudsen, and Anette Sundbye. Karin Westrum processed all samples in the lab to extract mites and prepare subsamples of specimens suitable for identification. The mounting and further identification was performed at the Laboratory of Acarology, ESALQ (University of São Paulo, Brazil), by Gilberto J. de Moraes and Joao Pedro I. Martin. Nina Trandem has led the work and written most of the report.

Voucher specimens from the study are kept at NIBIO and the Laboratory of Acarology, ESALQ.

Ås, 3rd of December 2020

Anette Sundbye

Innhold

1 Introduction and background

1.1 Why look for *Amblyseius andersoni* in Norway?

Predatory mites of the family Phytoseiidae (Acari: Mesostigmata) are well-known beneficial organisms, widely used in biological control of small arthropods like spider mites, thrips and whiteflies. In Norway, four phytoseiid species are presently commercially available, whilst 12 species are available in the EU (EPPO 2019). In 2012, researchers, advisors and retailers working with biological control in Norway were asked to list biocontrol products available in EU which would be useful also in Norway. The predatory mite *Amblyseius andersoni* Chant was high on the resulting list for fruit and berry crops (Sundbye et al. 2014). This species is available in the EU, where it has been used for control of various phytophageous mites (Tetranychidae, Eriophyidae, Tarsonemidae) in both outdoor and indoor crops since 2006 (EPPO 2019). A product containing *A. andersoni* is registered in both Sweden and Denmark. This has also been applied for the Norwegian market, and a national risk assessment for the species has recently been carried out (VKM et al. 2020).

The phytoseiid fauna of Norway is relatively well known, with 62 species listed in the most recent checklist (Gwiazdowicz and Gulvik 2005). This is mainly due to the work of Torgeir Edland (1932-1999), who collected phytoseiids on plants from the whole country (e.g. Edland 1987) and published the results in collaboration with taxonomists (e.g. Karg and Edland 1987, Edland and Evans 1998; Denmark and Edland 2002).

Edland never found *A. andersoni*. However, in 2016, considering the agronomic interest in the species and an increasingly milder climate in Norway (e.g. Rivero et al. 2016), we found it worthwhile to carry out a specific search for *A. andersoni* about two decades after Edland's last studies. We here report the results from this search. The main results have also been used in the recent risk assessment (VKM et al. 2020).

1.2 Distribution and plant substrates of *A. andersoni*

In general, *A. andersoni* has a Holarctic distribution (Demite et al 2020). It has been reported as naturally occurring in several countries in North Europe, including UK (e.g. Fitzgerald and Solomon 2002), Denmark (Hansen and Johnsen 1986) and Sweden (Steeghs et al 1993), but not Norway and Finland. The finds in UK have been in England, and the ones in Sweden in Scania, i.e. in southern parts of the countries, only.

A frequent plant substrate reported is *Malus domestica* (apple trees). This probably reflects a true habitat preference of the species but also that pome orchards have been among the most intensively surveyed habitats for Phytoseiidae in general, due to the development of integrated pest management in pome fruit production since the 1960's (Blommers 1994, Edland 1995). Apart from apple trees, *A. andersoni* has been found naturally occurring on 5 plant species in Denmark (Hansen and Johnsen 1986) and 6 in Sweden (Steeghs et al. 1993).

1.3 Objectives

The main objective of this study was to selectively search for *A. andersoni* - maximizing the probability of finding it - with the resources and information available.

2 Materials and methods

2.1 Collecting mites

Since T. Edland never found *A. andersoni* during his large sampling effort on Phytoseiidae in fruit orchards, we decided to rather focus on blackberry. Blackberry is the only plant, apart from apple, on which *A. andersoni* has been found in both Sweden and Denmark (listed as *Rubus fruticosus* in Steeghs et al 1993, and *R. plicatus* in Hansen and Johnsen 1986). We therefore sampled leaves from uncultivated ('wild') blackberry plants, i.e. plants of *Rubus fruticosus* (sensu lato). In Norway, about 40 species of wild blackberry have been found in total, they are all closely related and difficult to distinguish (Espedal 2002). They are typically found in boundary vegetation, along roads or agricultural fields, or in other open vegetation types (Figure 1).

Figure 1. Two of the localities used for sampling blackberry leaves. (Photo Geir K. Knudsen and Karin Westrum.)

In Norway, blackberry only grows in climatically favourable localities in the south, mostly along the coast (Espedal 2002). This is also where we would expect *A. andersoni* to occur should it be present in Norway. The sampling protocol also specified that whitebeam (*Sorbus intermedia, S. norvegica*, etc) should be sampled if present in a blackberry locality, as *A. andersoni* was reported in Sweden on *S. intermedia* (Steeghs et al 1993).

The areas selected for sampling were in the vicinity of Sandefjord, Grimstad, Ås and Fredrikstad, respectively, all in southeast Norway (exact coordinates are given in Results). To collect as many mites as possible, the sampling was done towards the end of the summer.

Each leaf sample consisted of about 15 fullly grown leaves put in a paper bag. The paper bags were put in plastic bags and kept at fridge temperature until each sample could be put in a 3L container with lukewarm water in the laboratory at Ås. The water had been added three drops of soap to prevent dislodged arthropods to float, and each sample was left for ca. 24 hrs before filtering the water through a sieve with mesh size 160 µm. All material in the sieve was put in 70 % alcohol for later examination. A detailed description of this leaf washing process is given in Nordengen and Klingen (2006). A similar washing method was also used by Edland (cf. Edland and Evans 1998).

2.2 Identification of mites

During the autumn of 2016, a subsample of specimens assessed to be adult phytoseiids under a stereo microscope, were picked from each leaf sample and sent to the Laboratory of Acarology at ESALQ, University of Sao Paulo, Brazil, for identification. Further subsamples of adult mites were brought to ESALQ by Gilberto José de Moraes, who visited Norway in January 2018. At the Acarology lab, the

specimens were mounted on microscope slides in in Hoyer´s medium. The slides were maintained in an oven (at about 60 ^{o}C) for about 10 days, for clearing and drying.

The actual identification was done under optical phase contrast (Leica, DMLB) and interference contrast (Nikon, Eclipse 80i) microscopes, at 400 and 1000 magnification. Initially, adults were separated from other stages, as usually identification to species can only be done by the examination of adult mites. These mites were initially identified to genera using an unpublished dichotomous key regularly used for the taxonomy of Phytoseiidae at The Summer Acarology Program of the Ohio State University (USA). Species of each genus were identified by comparison with specimens available in the mite collection of the Acarology Laboratory of ESALQ-USP, or with descriptions and redescriptions available in the world literature. The specimens were first compared with species previously reported in Norway and surrounding countries, and only if not found in those places, comparisons would be conducted with descriptions of species from other parts of the world. Of fundamental importance in this process was the “Phytoseiidae Database” (Demite et al 2020). Upon detection of closely related species, comparisons would involve shape and measurements of different structures, mainly chelicera, dorsal and ventral shields, setae, spermatheca and leg macrosetae. Measurements were done with a graded ocular attached to the interference contrast microscope.

3 Results and discussion

In total, 37 leaf samples of blackberry (ca. 550 leaves in total) and 3 of whitebeam (45 leaves) were collected at 15 localities during August 2016. Phytoseiid mites were quite common: almost 1500 specimens of potential phytoseiids were retrieved from blackberry leaves with the washing method, giving an average density of 2.7 such mites per leaf (all mobile stages included). On whitebeam, which has smaller leaves, the corresponding density was 2.0 per leaf.

About two thirds of the potential phytoseiids were sent for identification, but not all specimens could be successfully retrieved after the dispatchment, and some were subadult. Altogether 544 specimens (538 from *Rubus* and 6 from *Sorbus*) were identified to species or genus level. The following 10 phytoseiids were found:

- *Euseius finlandicus* (Oudemans, 1915)
- *Kampinodromus* sp. (only juveniles found)
- *Paraseiulus soleiger* (Rigaba, 1904)
- *Phytoseius spoofi* (Oudemans, 1915)
- *Typhlodromus (Anthoseius) caucasicus* (Abbasova, 1970)
- *Typhlodromus (Anthoseius) caudiglans* Schuster, 1959
- *Typhlodromus (Anthoseius) halinae* (Wainstein & Kolodochka, 1974)
- *Typhlodromus (Anthoseius) parinopinatus* (Evans & Edland, 1998)
- *Typhlodromus (Anthoseius) rhenanus* (Oudemans, 1905)
- *Typhlodromus (Typhlodromus) pyri* Scheuten, 1857

Hence, *Amblyseius andersoni* was not found, nor any other *Amblyseius* species. Details on locations, sampling effort and species on blackberry leaves are given in Table 1, and for whitebeam in Table 2. All the 9 species listed have been found in Norway earlier, and also two species of *Kampinodromus* (Demite et al 2020). The Norwegian checklist (Gwiazdowicz and Gulvik 2005) does not include *P. spoofi*, but this species is definitely present in Norway (Edland 1993, Hossain 1995).

The two most numerous species on blackberry were *T.* (*A.*) *rhenanus* and *T.* (*T.*) *pyri*, both well-known in Norway (Edland 1987). Although not as intensively studied as fruit orchards, Edland also sampled blackberry (*R. fruticosus*), on which he reported 10 species: *Amblyseius januaricus* Wainstein & Vartapetov, *Typhlodromus* (*Anthoseius*) *bakeri* (Garman), *T.* (*A.*) *caucasicus*, *T.* (*A.*) *foenilis* Oudemans, *T.* (*A.*) *rhenanus*, *T.* (*A.*) *suecicus* Sellnick, *Neoseiulus scoticus* (Collyer), *N. umbraticus* (Chant), *Neoseiulella sexapori* (Karg & Edland), and *T.* (*T.*) *pyri* (Karg and Edland 1987, Edland and Evans 1998, Evans and Edland 1998, Denmark and Edland 2002). Thus, in total, 17 phytoseiid species have now been found on blackberry in Norway. It is interesting to note that there is an overlap of only three species between our limited 2016 survey and Edland's studies.

In a similar study conducted in southern Brazil on *Rubus rosifolius* (thimbleberry/ roseleaf bramble) in 2013-2014, eight phytoseiid species were found (Zanfelici, 2020), namely *Amblyseius operculatus* De Leon, *Euseius alatus* DeLeon, *Euseius ho* De Leon, *Iphiseiodes* sp., *Neoseiulus tunus* (De Leon), *Phytoseiulus macropilis* (Banks), *Phytoseius woodbury* Muma & Denmark, and *Proprioseiopsis* sp. This is basically a completely different group of species, occurring on a similar plant species, as expected from the very different ecological conditions prevailing in Norway and southern Brazil. The most outstanding difference refers to the common occurrence of *Typhlodromus* (*Anthoseius*) species

in Norway, and their total absence in southern Brazil. Species of this subgenus are found in Brazil (Demite et al., 2020) on other plant species, but they are much less common than in Norway.

On whitebeam, our sample effort was small (Table 2), and both species found there were also present on blackberry. Edland did not specifically report any phytoseiids on whitebeam.

Table 1. Sampling effort and phytoseiid species identified on leaves of wild blackberry, *Rubus fruticosus* (sensu lato). Each leaf sample consisted of 15 fully grown leaves. Maximim numbers of phytoseiids are all mobile stages of mites assessed as potential phytoseiids under a stereo microscope at NIBIO.

AREA (Sampling date) Site* (No of samples)	UTM32 coordinates (East, North)	Max. no of Phytoseiidae	Phytoseiid species found in subsamples sent for identification (full names given on page 10)
SANDEFJORD (19 and 21 Aug 2016)			
1:Himberg lawn (2)	564973, 6552134	93	*Typhlodromus halinae*, *T. parinopinatus*, *T. rhenanus*, *Phytoseius spoofi*
2:Himberg clearcut (3)	564873, 6552048	63	*Typhlodromus halinae*, *T. parinopinatus*
3:Haugen embank. (3)	564829, 6551969	161	*Typhlodromus rhenanus, T. halinae*, *T. parinopinatus*
4:Haugen W embank(3)	564823, 6551913	36	*Typhlodroumus halinae*
5:Vøra (2)	572130, 6548521	95	*Typhlodromus caucasicus, T. halinae, T. pyri, Paraseiulus soleiger*
6:Vøra cottage (3)	572238, 6548927	221	*Typhlodromus rhenanus, T. pyri, T. caudiglans, T. hyalinae, T. parinopinatus, Euseius finlandicus*
GRIMSTAD (14 Aug 2016)			
7:Tromøy Løken (3)	495152, 6484863	86	*Typhlodromus rhenanus, T. pyri, T. caucasicus, T. parinopinatus*
8:Tromøy Kjørviga (3)	495849, 6485058	90	*Typhlodromus caucasicus, T. caudiglans, T. halinae, T. pyri, T. rhenanus*
10:Tromøy Skare (3)	494198, 6482492	218	*Typhlodromus rhenanus, T. pyri, Kampinodromus* sp***, T. caucasicus, T. halinae*
11:Tromøy Bjelland (3)	493558, 6480341	ca. 50	*Typhlodromus rhenanus, T. pyri*
12:Tromøy Spornes (3)	491720, 6478300	121	*Typhlodromus pyri, T. parinopinatus, T. halinae*
FREDRIKSTAD (21 Aug 2016)			
13:Lilleng (1)	604043, 6566499	42	*Typhlodromus halinae, Phytoseius spoofi*
14:Lerberget/Tyrjell (1)	604962, 6567055	21	*Typhlodromus parinopinatus*
ÅS (30 Aug 2016)			
15:Kaja water tap (3)	599978, 6615853	176	*Typhlodromus rhenanus, T. halinae*

*The number preceding each site name is the reference number used for samples from that site during the study.

**Juveniles only (cannot be identified to species).

Table 2. Sampling effort and phytoseiid species identified on leaves of Sorbus (found in one of the sites, only). Each leaf sample consisted of 15 fully grown leaves. Maximum number of Phytoseiidae is all mobile stages of mites assessed as potential Phytoseiidae under a stereo microscope at NIBIO.

AREA (Sampling date) Site* (No of samples)	UTM32 coordinates (East, North)	Max. no of Phytoseiidae	Phytoseiid species found in subsamples sent for identification (full names given on page 10)
GRIMSTAD (14 Aug 2016)			
9:Tromøy Kjørviga (3)	495849, 6485058	90	*Typhlodromus caucasicus, T. pyri*

*The number preceding the site name is the reference number used for samples from that site during the study.

4 Conclusions

Our results support the absence of *Amblyseius andersoni* in earlier Norwegian surveys of Phytoseiidae, indicating that the species either lacks the ability to establish under current Norwegian outdoor conditions or has not yet dispersed to Norway. It should be noted that the sampling effort in the surveys published from Sweden and Denmark, where the species was found, was considerably less than the total sampling effort made in Norway.

If any further search for naturally occurring *A. andersoni* is to be done, we would suggest to look in the county of Rogaland, which has a mild climate, and once there, to check for its possible presence in costal dunes, which has been reported as a typical habitat for the species in Latvia (Salmane and Petrova 2002). Also, it would seem worthwhile to look for it on plants with glabrous (hairless) leaves. As summarized by McMurtry et al. (2013), this is the typical type of habitat favoured by *A. andersoni*. The same authors classified *Amblyseius* species as generalist predators living on glabrous leaves.

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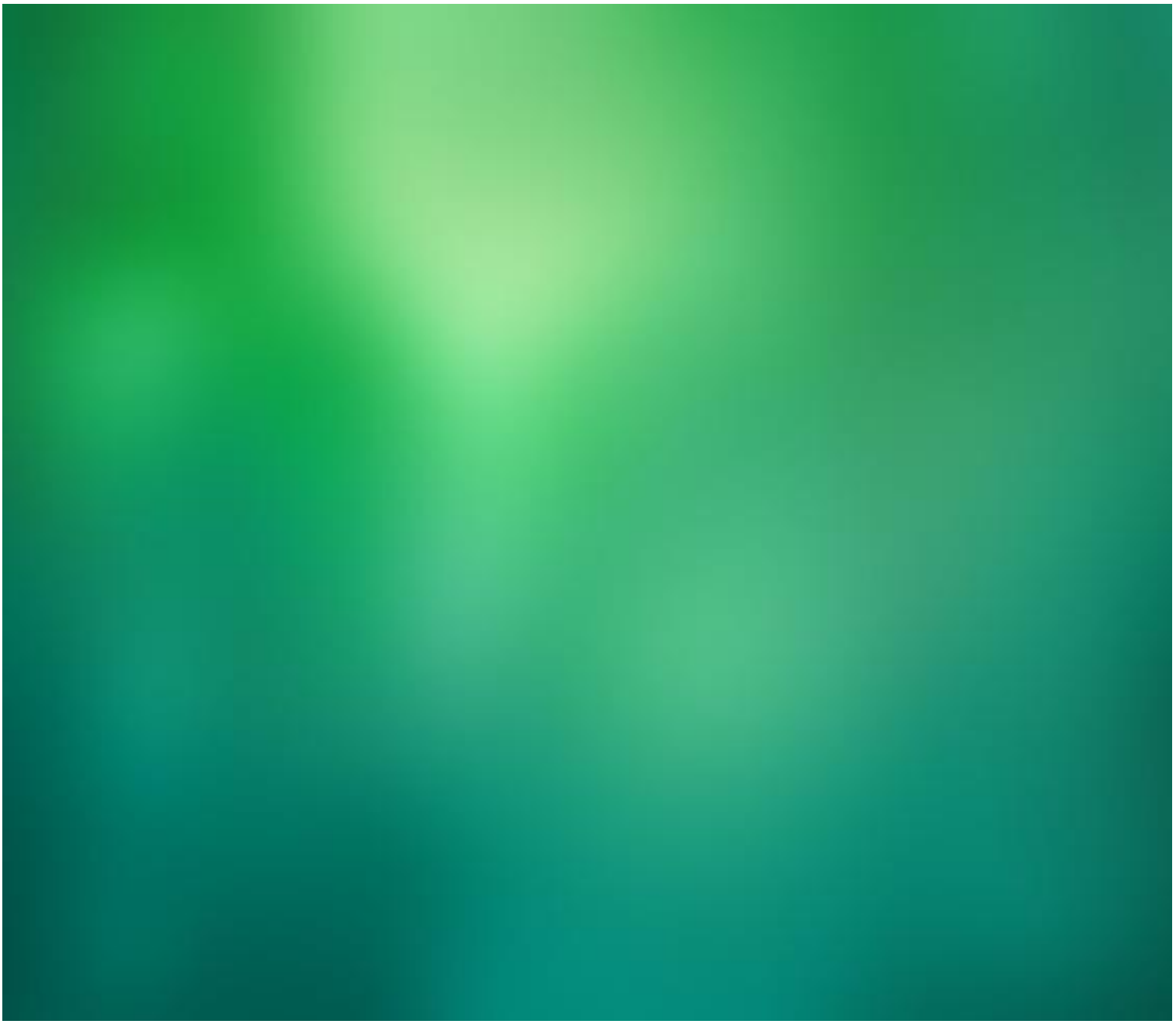

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